

BioChek B.V.



PRODUCT CATALOGUE

General Information on BioChek products

September 2002

BioChek B.V.

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Available products:

ELISA kits* for most common diseases, easy to use, short incubation times, high quality standard, same diluent all kits.

Reference controls additional pre-diluted controls for quantitative assays.
RF4: titre level inactivated vaccination

Microtiterplate reader*: Fast and accurate, low maintenance instrument. Will read all 96 wells in less than a few seconds.
Computer controlled.

Precision pipettes: single channel 1 microliter pipette and multichannel , 8 or 12 channel, pipettes. Best quality for optimal results

Washing system: either manual or automatic systems. For consistent results we recommend an automatic system.

BioChek 2000 software: Windows software, visual set-up, very easy to use. Allows for on screen data analysis, export of data to Microsoft programs like excel, word, etc. E-mail data files to other BioChek users. In addition there is an automatic data interpretation option.

Technical support: please contact us for support required. We provide training for lab- technicians in our facilities or on site at the customer.

* see following pages for details

Ship to:

Order No:

Date:

Ordered by:

PRODUCTS	Species		PRODUCT CODE	Size (Kits)	No. of kits price/kit		Total/kit
	C=Chickens	T=Turkeys					
	* not validated for turkeys						
Infectious Bursal Disease (Ab)	C	IBD	CK113	480 Tests			
Newcastle Disease (Ab)	C/T	NDV	CK116	480 Tests			
Infectious Bronchitis Virus (Ab)	C	IBV	CK119	480 Tests			
Reovirus (Ab)	C	REO	CK110	480 Tests			
Avian Rhinotracheitis (Ab) (strips)	C/T	ART	CK120	480 Tests			
Egg Drop Syndrome (Ab)	C	EDS	CK112	480 Tests			
Mycoplasma Gallisepticum (Ab)	C/T	MG	CK114	480 Tests			
Mycoplasma Synoviae (Ab)	C/T	MS	CK115	480 Tests			
Mycoplasma Meleagridis (Ab)	T	MM	CK109	480 Tests			
Mycoplasma Gallisepticum-Synoviae (Ab)	C/T	MG/MS	CK215	480 Tests			
M.G./M.S. 20 plate kit	C/T	MG/MS	CK230	1920 Tests			
M.G./M.S./EDS (Ab)	C	TRIPLE	CK319	480 Tests			
Salmonella Enteritidis (Ab)	C/T	SE	CK117	480 Tests			
Salmonella Typhimurium Ab	C/T*	ST	CK118	480 Tests			
Salmonella Enteritidis/S. Typhimurium (A)	C/T*	SE/ST	CK218	480 Tests			
S.E/S.T. 20 plate kit	C/T*	SE/ST	CK220	1920 Tests			
Ornithobacterium Rhinotracheale (Ab)	C/T	ORT	CK108	480 Tests			
Avian Leukosis Virus 5 plate kit (Ag)	C	LLAG	CK422	480 Tests			
Avian Leukosis Virus 50 plate kit (Ag)	C	LLAG	CK111	4800 Tests			
Avian Influenza	C	AI	CK121	480 Tests			
Avian Encephalomyelitis(strips)	C/T	AE	CK123	480 Tests			
Infectious Laryngotracheitis(strips)	C	ILT	CK124	480 Tests			
Reticuloendotheliosis	C/T	REV	CK125	480 Tests			
Other products							
reference control(High titre for IBD/IBV/NDV/REO/ART/AE(=TRT)			RF04	5 ml			
Shipping and handling							

	Units		
Total			

Key features of available kits:

General

During the development stage of a new ELISA kit submit our kits to a very stringent evaluation in order to assure that sensitivity, specificity and differentiating capabilities of our kits meet or exceed the demands of our users. During the production of a kit, besides a QC on above criteria, we strongly focus on reproducibility of our kits. In order to enable our customers to monitor the reproducibility of our test we have available a reference control (R4). R4 is a pre-diluted sample with a fixed expected titre range.

Most kits will detect positive samples 7 - 14 days post infection. Most kits can be used for chickens and Turkeys.

Quantitative Antibody detection Kits

IBD	Excellent correlation to VN. Differentiates in titre levels from low to high (VN 7 - 18/19). Validated for vaccination date prediction in Broilers and Layer Breeders.
NDV	excellent correlation with HI suitable for Chicken and Turkey sera.
IBV	excellent correlation with HI, detects most known serotypes
REO	Good correlation with other available tests
EDS	Good correlation with HI, very specific for EDS antibodies, doesn't detect other ADENO viruses in Poultry
ART(=TRT)	Detects both A and B strain. Good sensitivity
ORT	detects ORT strain A to G same kit for Chickens and Turkeys
AE	Good correlation with other available tests
ILT	highly specific and a good sensitivity.
REV	highly specific and a good sensitivity

Qualitative Antibody detection Kits

Mycoplasma's (MS/MG ; Mg,Ms and Mm)	Excellent sensitivity and specificity the combination test can be used for screening, when positive use separate Mg and Ms for determination of Mg or Ms.
Salmonella's (Se/St ; Se and St)	Excellent sensitivity and specificity the combination test can be used for screening, when positive use separate Se or St for serological confirmation.
Avian Influenza	detect antibodies to group A avian influenza. When positive use AGP or HI test to confirm.

Qualitative Antigen detection Kits

Avian Leukosis(P27) test	Excellent sensitivity and specificity, will detect P27 common Leucosis antigen. Validated for Albumen, Cloacal Swabs and Meconium testing.
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BioChek technical fact sheet

Following are the technical details of the BioChek flock health monitoring system.

Computer:

Minimum Hardware requirements:

IBM compatible 486 DX, 16 MB RAM, hard disk, colour screen 800 x 600 pixels, minimum 20 MB available space on hard disk.

1 free serial port (in addition to mouse port)

Printer

Software requirements:

Windows: 3.11 or W 95, W98, etc.or Windows NT

Readers:

Readers interfacing with BioChek software

SLT Spectra, Sunrise, EASY, E-max, BioTech 308, 311 & 312, ELX800, Dynatech MR 600 - 7000 (internal software version 3.7 required), Dynatech MRX, Dynatech DIAS , Multiscan +, MCC and MS, Anthos HT.

(this list gets updated regularly, please contact us if your reader isn't on the list!!)

ELISA tests:

5 plates per kit, 2 negative and 2 positive controls per plate.

Most run at a 1:500 dilution, at room temperature. Tests read at 405 NM, shelflife 1 year after manufacturing, all reagents provided. Qualitative and semi-quantitative antibody tests.

The kits are designed to cover the needs for practical serology. In general our kits will detect a positive sample 7 - 14 days after infection/vaccination and the range will comfortably cover live and inactivated vaccination titre levels.

Key features of Software

GENERAL

The BioChek software is designed for easy visual recognition. This makes the software *very easy to use*. Reports are standardised and allow for either producing individual results as reports allowing for up to 20 flocks per page.

MENU'S

There are 3 menus READ, REPORTS and CONFIG. In the READ menu one does everything which has to do with reading and data saving, in the REPORT menu one does everything which has to do with generating reports. In the CONFIG menu one may customise settings to meet own requirements.

FLOCK CODING SYSTEM:

18 different fields can be chosen:

(in bold are the fields mostly selected)

name	brand of chicken
company	vaccination code
code	manager
area	veterinarian
region	technician
complex	flock performance
age	reason of testing
type of chicken	house number
placement	

The relevance of this coding system is:

- information will be printed on report
- flocks can be retrieved on any field or combination of fields. This allows for comprehensive statistics(see reports)
- automatic data interpretation linking expected titre values to age and type of poultry in flockcode.

Specific features:

Data Input/Reading plates

Layout template: After saving results template will remain on screen for next assay on same samples. Layout can also be saved for future use.

Layout orientation: vertical or horizontal sample orientation can be selected.

Controls: can be moved over the by dragging with the mouse to any position.

EDIT results: when errors have been made in the code simply use edit button and correct.

Reports:

*

reports allowing for comment addition

- General:** When setting up target titer option in the config menu, all histogram reports will appear with a target titer and %CV range compared to actual titer and %CV. See example on page 8.
- Today's results*:** Prints tests done same day, one can print on letterhead, add comments, print. Will use one page for all assays done on samples with same code.
- Vaccination date prediction:**
One can select out of 3 methods. Square root calculation, Log2 calculation and the Deventer method.
Select method in configuration menu. We recommend the "Deventer" method.
- Baseline calculation:** This report will calculate the mean titre over selected flocks. It will also calculate the minimum and the maximum titre of these flocks.

The benefit of this report is that one can easily calculate what titres are average at a certain age of a flock. For example. You want to know what titre level is "normal" at say 24 - 27 weeks of age in certain flocks. All you have to do is input these criteria and you'll get your average.
- Trend:** will calculate baseline per month. Bar graph X-axis month/year; Y-axis mean titre per month
- numerical summary** with this report one gets the results (mean titre, %CV, no of samples negative, positive and suspect and number of samples per titre group. Maximal 19 flocks per page.
- Flocks over time** generates a bar graph for selected flocks with age on X-axis and mean titre on Y-axis
- Blockdiagram/histogram*:**
single flocks presented either with OD values and calculated values or presented as a visually attractive histogram or a combination of both. For histograms one can select multiple histograms/page (max. 8 histograms/page) or 1 flock/page (all assays same flock on 1 page) comments can be added.
- Multiple results:** This is a numerical report showing: mean titre, %CV, mean negative control, mean positive control and titre per sample. Around 16 flocks per page.
- Compare to target:** will calculate the percentage of flocks with mean titre and CV in target, under target and over target see example page
- QC report:** For tracking additional control sera. Will plot the lot number of the kit on the X-axis and the obtained titre on the Y-axis.
- Custom report*:** select your own criteria for a report. Save under your own name, and use from then on !

Generating reports:

When leaving all fields of the flockcode open (name, company etc.) all flocks for selected period will be found. The more one enters into the code fields the more one narrows down the search.

Baseline Titer Calculations

Name:

Company:

Code:

Birthdate:

Age:

House no:

Reason test:

Type chicken:

Internal control:

Bleeding date:

Closest match searched flock codes:

Find...

Date	Name:	Company:	Co

Parameters

Period: <->

Age: <->

Assay's:

- Assay
- Gumboro Disease
- EDS
- NDV
- St

PRINT Cancel

The baseline report(mean titre all selected flocks + minimum and maximum titer) will be calculated on the flocks with the following criteria:

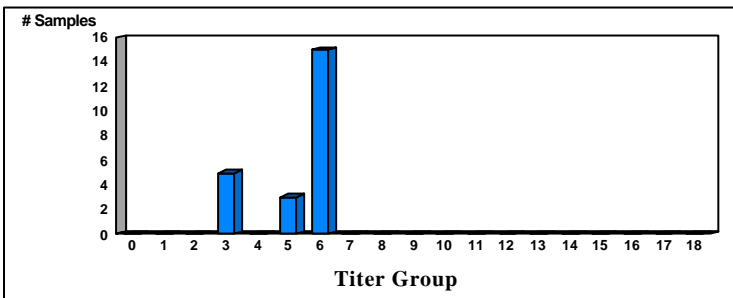
Name: any
Company: Bio (all companies with first 3 letters bio)
code: any
birthdate any
age between 22W(eeks) and 26W, see parameters
house No any
Reason test: any
Type of Chicken: BB (Broiler Breeders)
Bleeding data: between 01-01-1998 and 04-11-1998 (see parameters)
Assays IBD and NDV

Click on print and the report will be made. In a similar manner all other reports can be generated.



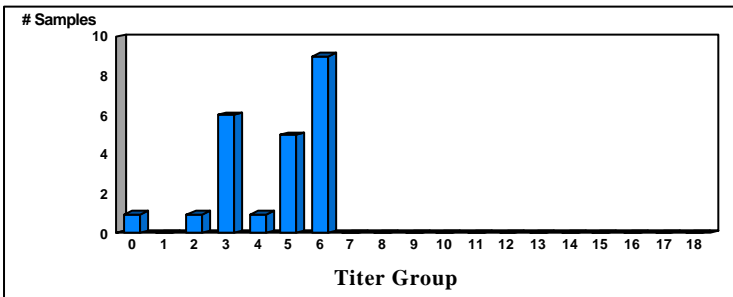
Flock:

Name : TEST
 Company : BIO
 Code : 1234
 Age : 30W
 House No. : 01
 Reason for Testing: SCREEN
 Type of Bird : BB
 Bleed Date : 23/11/2000



Assay : IBD
 Bleeding Date : 23/11/2000
 Samples : 23

Mean Titer : 4004
 GMT: 3673
 %CV : 35
 Target Titer: 7 000 - 25 000
 Target %CV: 20 - 45
 Interpretation Titer: LOW
 Interpretation CV: OK



Assay : NDV
 Bleeding Date : 23/11/2000
 Samples : 23

Mean Titer : 4773
 GMT: 3672
 %CV : 47
 Target Titer: 10 000 - 25 000
 Target %CV: 20 - 45
 Interpretation Titer: LOW
 Interpretation CV: HIGH

PM

Nothing to report

Interpretation

NDV titers are poor.

Advise

Consider revaccination of NDV



Demo version

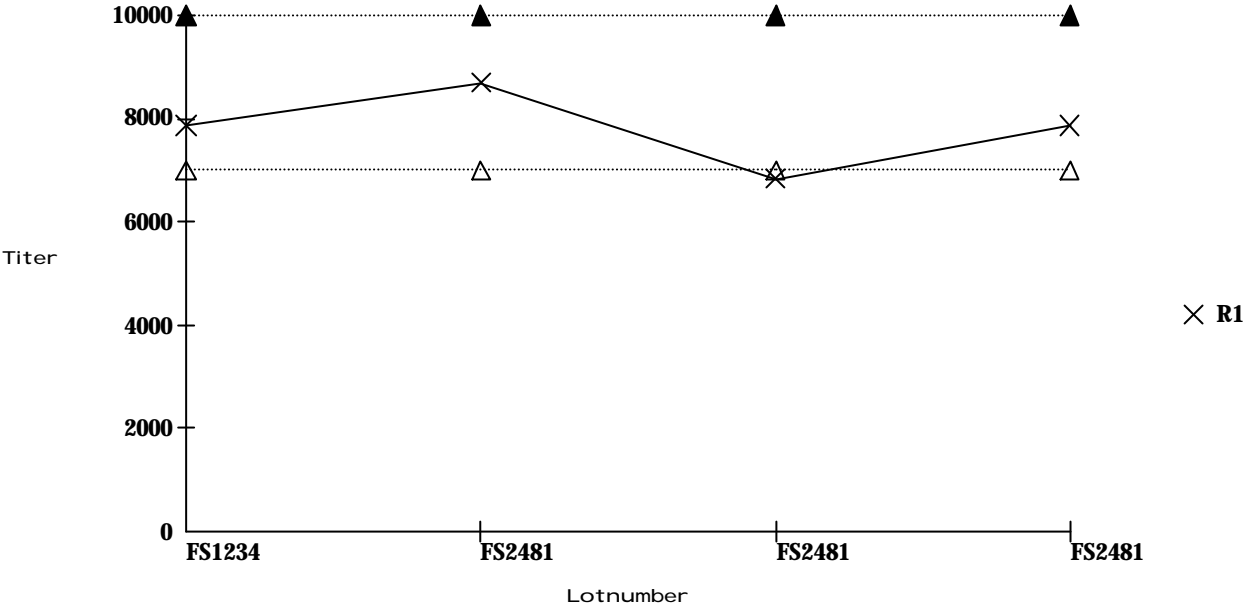
Report: Compare To Target

This reports compares the Target (desired) Mean Titers & %CV to the Obtained Mean Titers & %CV

Period: 01/01/2000 <-> 23/11/2000

<u>Test</u>	<u>Type</u>	<u>Age Interval</u>	<u>Target Mean Titer Range</u>	<u>No.Flocks</u>	<u>Mean Flock Titers Compared to Target</u>			<u>% CV of Flocks in Target Range</u>		
					<u>% In Target</u>	<u>% Above Target</u>	<u>%Under Target</u>	<u>%CV OK</u>	<u>%CV High</u>	<u>%CV Low</u>
IBD	BB	24W - 60W	7000- 25000	92	11	0	89	11	0	0
NDV	BB	24W - 60W	10000- 25000	3	0	0	100	0	0	0
NDV	BB	5W - 23W	4000- 10000	4	100	0	0	100	0	0

Quality Control



BioChek Poultry Immunoassays

Infectious Bursal Disease Antibody Test Kit

Catalogue Code CK113

Description of Test

The IBD ELISA kit will measure the amount of antibody to IBD in the serum of chickens. Microtitre plates have been pre-coated with inactivated IBD antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-IBD antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti-IBD antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti-IBD antibody is present and the intensity is directly related to the amount of anti-IBD present in the sample.

Reagents provided

1. **IBD Coated plates.** Inactivated viral antigen on microtitre plates
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to IBD in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLETS WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22 - 27 °C) before use.

Sample preparation

Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove IBD coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and samples by reading at 405 nm.

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.2.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The IBD positive control has been carefully standardised to represent significant amounts of antibody to IBD in Chicken serum.

The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .2 or greater contain anti-IBD antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

2. Calculation of Antibody Titre

The following equation relates the S/P of a samples at a 1 : 500 dilution to an end point titre

$$\text{Log}_{10} \text{Titre} = 1.1 * \text{Log}(\text{SP}) + 3.361$$

$$\text{Antilog} = \text{Titre}$$

S/P value	Titre Range	Antibody status
.149 or less	284 or less	Negative
.150 - 0.199	285 - 390	Suspect
.200 or greater	391 or greater	Positive

Each Laboratory should establish its own criteria for non protected and protected

BioChek has available a software programme which can be used with the IBD kit to calculate S/P values, titres and provide general flock profiling.

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BioChek Poultry Immunoassays

Newcastle Disease Antibody Test Kit

Catalogue Code CK 116

Description of Test

The NDV ELISA kit will measure the amount of antibody to NDV in the serum of chickens. Microtitre plates have been pre-coated with inactivated NDV antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-NDV antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti-NDV antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti-NDV antibody is present and the intensity is directly related to the amount of anti-NDV present in the sample.

Reagents provided

1. **NDV Coated plates.** Inactivated viral antigen on microtitre wells.
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to NDV in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLETS WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22-27°C) before use.

Sample preparation

Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove NDV coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and the samples by reading at 405 nm.

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.2.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The NDV positive control has been carefully standardised to represent significant amounts of antibody to NDV in Chicken serum.

The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .350 or greater contain anti- NDV antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

2. Calculation of Antibody Titre

The following equation relates the S/P of a samples at a 1 : 500 dilution to an end point titre

$$\text{Log}_{10} \text{Titre} = 1.0 * \text{Log}(\text{SP}) + 3.52$$

$$\text{Antilog} = \text{Titre}$$

S/P value	Titre Range	Antibody status
.249 or less	827 or less	Negative
.250 - 0.349	828 - 1158	Suspect
.350 or greater	1159 or greater	Positive

Each Laboratory should establish its own criteria for non protected and protected

BioChek has available a software programme which can be used with the NDV kit to calculate S/P values, titres and provide general flock profiling.

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BioChek Poultry Immunoassays

Infectious Bronchitis Antibody Test Kit

Catalogue Code CK119

Description of Test

The IBV ELISA kit will measure the amount of antibody to IBV in the serum of chickens. Microtitre plates have been pre-coated with inactivated IBV antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-IBV antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti-IBV antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti-IBV antibody is present and the intensity is directly related to the amount of anti-IBV present in the sample.

Reagents provided

1. **IBV Coated plates.** Inactivated viral antigen on microtitre plates
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to IBV in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLET WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22 – 27 °C) before use.

Sample preparation

Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove IBV coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and the samples by reading at 405 nm.

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.2.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The IBV positive control has been carefully standardised to represent significant amounts of antibody to IBV in Chicken serum.

The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .2 or greater contain anti-IBV antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

2. Calculation of Antibody Titre

The following equation relates the S/P of a samples at a 1 : 500 dilution to an end point titre

$$\text{Log}_{10} \text{Titre} = 1.0 * (\log_{10} \text{S/P}) + 3.62$$

Antilog = Titre

S/P value	Titre Range	Antibody status
0.149 or less	624 or less	Negative
0.150 - 0.199	925 - 833	Suspect
.200 or greater	834 or greater	Positive

Each Laboratory should establish its own criteria for non protected and protected

BioChek has available a software programme which can be used with the IBV kit to calculate S/P values, titres and provide general flock profiling.

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BioChek Poultry Immunoassays

Avian Reovirus Antibody test Kit

Catalogue Code CK110

Description of Test

The REO ELISA kit will measure the amount of antibody to REO in the serum of chickens. Microtitre plates have been pre-coated with inactivated REO antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-REO antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti- REO antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti-REO antibody is present and the intensity is directly related to the amount of anti- REO present in the sample.

Reagents provided

1. **REO Coated plates.** Inactivated viral antigen on microtitre wells.
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to REO in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLET WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22-27°C) before use.

Sample preparation

Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove REO coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and the samples by reading at 405 nm.

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.2.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The REO positive control has been carefully standardised to represent significant amounts of antibody to REO in Chicken serum.

The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .200 or greater contain anti- REO antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

2. Calculation of Antibody Titre

The following equation relates the S/P of a sample at a 1 : 500 dilution to an end point titre

$$\text{Log}_{10} \text{Titre} = 1.1 * (\text{log}_{10} \text{S/P}) + 3.9$$

$$\text{Antilog} = \text{Titre}$$

S/P value	Titre Range	Antibody status
.149 or less	985 or less	Negative
.150 - 0.199	986 - 1351	Suspect
.200 or greater	1352 or greater	Positive

Each Laboratory should establish its own criteria for non protected and protected flocks

BioChek has available a software programme which can be used with the REO kit to calculate S/P values, titres and provide general flock profiling.

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BioChek Poultry Immunoassays

Avian Rhinotracheitis Antibody Test Kit

For the detection of antibody to Avian Pneumovirus in Chickens and Turkeys

Catalogue Code CK120

Description of Test

The ART ELISA kit will measure the amount of antibody to ART in the serum of chickens and Turkeys. Microtitre plates have been pre-coated with inactivated ART antigen. Serum samples are diluted and added to the microtitre wells where any anti-ART antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti-ART antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti-ART antibody is present and the intensity is directly related to the amount of anti-ART present in the sample.

Reagents provided

1. **ART Coated plates.** Inactivated viral antigen(Avian pneumovirus) on microtitre plates
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to ART in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLETS WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22-27°C) before use.

Sample preparation

Dilute each test sample 1 : 500 by adding 1 µl to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove ART coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **60 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **60 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and the samples by reading at 405 nm.

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.2

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The ART positive control has been carefully standardised to represent significant amounts of antibody to ART in Chicken or Turkey serum.

The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .5 or greater contain anti-ART antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

2. Calculation of Antibody Titre

The following equation relates the S/P of a samples at a 1 : 500 dilution to an end point titre

$$\text{Log}_{10} \text{Titre} = 1.0 (\log_{10} \text{S/P}) + 3.52$$

$$\text{Antilog} = \text{Titre}$$

S/P value	Titre Range	Antibody status
.349or less	1158 or less	Negative
.350	1159 - 1655	Suspect
.500or greater	1656 or greater	Positive

Each Laboratory should establish its own criteria for non protected and protected

BioChek has available a software programme which can be used with the ART kit to calculate S/P values, titres and provide general flock profiling.

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BioChek Poultry Immunoassays

Egg Drop Syndrom antibody test Kit

Catalogue Code CK112

Description of Test

The EDS ELISA kit will measure the amount of antibody to EDS in the serum of chickens. Microtitre plates have been pre-coated with inactivated EDS antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti- EDS antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti-EDS antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti-EDS antibody is present and the intensity is directly related to the amount of anti- EDS present in the sample.

Reagents provided

1. **EDS Coated plates.** Inactivated viral antigen on microtitre plates
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to EDS in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLET WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22-27°C) before use.

Sample preparation

Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove EDS coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and the samples by reading at 405 nm.

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.2.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The EDS positive control has been carefully standardised to represent significant amounts of antibody to EDS in Chicken serum.

The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .5 or greater contain anti-EDS antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

2. Calculation of Antibody Titre

The following equation relates the S/P of a samples at a 1 : 500 dilution to an end point titre

$$\text{Log}_{10} \text{Titre} = 1.14 (\log_{10} \text{S/P}) + 3.156$$

$$\text{Antilog} = \text{Titre}$$

S/P value	Titre Range	Antibody status
.349 or less	432 or less	Negative
.350 - 0.499	433 - 649	Suspect
.500 or greater	650 or greater	Positive

Each Laboratory should establish its own criteria for non protected and protected

BioChek has available a software programme which can be used with the EDS kit to calculate S/P values, titres and provide general flock profiling.

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BioChek Poultry Immunoassays

Mycoplasma gallisepticum Antibody Test Kit

Catalogue Code CK 114

Description of Test

The Mg ELISA kit will measure the amount of antibody to Mg in the serum of chickens. Microtitre plates have been pre-coated with inactivated Mg antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-Mg antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti-Mg antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti-Mg antibody is present and the intensity is directly related to the amount of anti-Mg present in the sample.

Reagents provided

1. **Mg Coated plates.** Inactivated mycoplasma antigen on microtitre wells
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to Mg in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially bio hazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLET WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22-27°C) before use.

Sample preparation

Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove Mg coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and the samples by reading at 405 nm.

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.3.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The Mg positive control has been carefully standardised to represent significant amounts of antibody to Mg in Chicken serum.

The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .500 or greater contain anti-Mg antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

2. Calculation of Antibody Titre

The following equation relates the S/P of a sample at a 1 : 500 dilution to an end point titre

$$\text{Log}_{10} \text{Titre} = 1.1 (\log_{10} \text{S/P}) + 3.156$$

$$\text{Antilog} = \text{Titre}$$

S/P value	Titre Range	Antibody status
.349 or less	450 or less	Negative
.350 - 0.500	451 - 667	Suspect
.500 or greater	668 or greater	Positive

Suspect or positive samples

Additional conventional serological testing should be performed on any suspect or positive samples in order to obtain a confirmed positive diagnosis of *Mycoplasma gallisepticum* within a chicken flock.

BioChek has available a software programme which can be used with the Mg kit to calculate S/P values, titres and provide general flock profiling.

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BioChek Poultry Immunoassays

Mycoplasma synoviae Antibody Test Kit

Catalogue Code CK 115

Description of Test

The Ms ELISA kit will measure the amount of antibody to Ms in the serum of chickens. Microtitre plates have been pre-coated with inactivated Ms antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-Ms antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti-Ms antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed anti-Ms antibody is present and the intensity is directly related to the amount of anti-Ms present in the sample.

Reagents provided

1. **Ms Coated plates.** Inactivated mycoplasma antigen on microtitre wells.
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to Ms in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLET WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22-27°C) before use.

Sample preparation

Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove Ms coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and the samples by reading at 405 nm

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.3.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The Ms positive control has been carefully standardised to represent significant amounts of antibody to Ms in Chicken serum.

The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .5 or greater contain anti-Ms antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

2. Calculation of Antibody Titre

The following equation relates the S/P of a sample at a 1 : 500 dilution to an end point titre

$$\text{Log}_{10} \text{Titre} = 1.27 * (\log_{10} \text{S/P}) + 3.156$$

$$\text{Antilog} = \text{Titre}$$

S/P value	Titre Range	Antibody status
.350 or less	378 or less	Negative
.350 - 0.499	379 -593	Suspect
.500 or greater	594 or greater	Positive

Suspect or positive samples

Additional alternative testing should be performed on any suspect or positive samples in order to obtain a confirmed positive diagnosis of Mycoplasma synoviae within a chicken flock.

BioChek has available a software programme which can be used with the Ms kit to calculate S/P values, titres and provide general flock profiling.

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BioChek Poultry Immunoassays

Mycoplasma meleagridis Antibody Test Kit

Catalogue Code CK 109

Description of Test

The Mm ELISA kit will measure the amount of antibody to Mm in the serum of turkeys. Microtitre plates have been pre-coated with inactivated Mm antigen. Turkey serum samples are diluted and added to the microtitre wells where any anti-Mm antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any turkey anti-Mm antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti-Mm antibody is present and the intensity is directly related to the amount of anti-Mm present in the sample.

Reagents provided

1. **Mm Coated plates.** Inactivated mycoplasma antigen on microtitre wells.
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to Mm in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLET WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22-27°C) before use.

Sample preparation

Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove Mm coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and the samples by reading at 405 nm.

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.3.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The Mm positive control has been carefully standardised to represent significant amounts of antibody to Mm in turkey serum.

The relative amounts of antibodies in turkey samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .500 or greater contain anti-Mm antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

2. Calculation of Antibody Titre

The following equation relates the S/P of a sample at a 1 : 500 dilution to an end point titre

$$\text{Log}_{10} \text{Titre} = 1.1 (\log_{10} \text{S/P}) + 3.156$$

$$\text{Antilog} = \text{Titre}$$

S/P value	Titre Range	Antibody status
.349 or less	450 or less	Negative
.350 - 0.499	451 - 667	Suspect
.500 or greater	668 or greater	Positive

Suspect or positive samples

Additional alternative testing should be performed on any suspect or positive samples in order to obtain a confirmed positive diagnosis of Mycoplasma meleagridis within a turkey flock.

BioChek has available a software programme which can be used with the Mm kit to calculate S/P values, titres and provide general flock profiling.

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BioChek Poultry Immunoassays

Mycoplasma gallisepticum/synoviae Combined Antibody Test Kit

Catalogue Code CK 215

Description of Test

The Mg/Ms ELISA kit will measure the amount of antibody to Mg or Ms in the serum of chickens. Microtitre plates have been pre-coated with inactivated Mg and Ms antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-Mg or anti-Ms antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti-Mg or anti-Ms antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti-Mg or anti-Ms antibody is present and the intensity is directly related to the amount of anti-Mg or anti-Ms present in the sample.

Reagents provided

1. **Mg/Ms Coated plates.** Inactivated mycoplasma antigens on microtitre wells.
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to Mg and Ms in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLET WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22-27°C) before use.

Sample preparation

Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove Mg/Ms coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and the samples by reading at 405 nm

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.3.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The Mg/Ms positive control has been carefully standardised to represent significant amounts of antibody to Mg or Ms in Chicken serum.

The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .5 or greater contain anti-Mg/Ms antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

Samples with an S/P of 0.349 or less are considered NEGATIVE. Samples with an S/P of between 0.350 and .499 are considered as SUSPECT and should be re-tested

Because the Mg/Ms combined test is a screening assay, it is not possible to generate antibody titre values that are valid for Mg or Ms.

The S/P generated for a sample will be for Mg or Ms. There is no differentiation between the two by this test.

For a general titre value, the following equation may be used:

$$\text{Log}_{10} \text{Titre} = 1.1 * \text{Log}(\text{SP}) + 3.156$$
$$\text{Antilog} = \text{Titre}$$

For identification of a positive sample, it will be necessary to run the sample again using a monospecific Mg kit (cat. No. CK 114) or Ms (cat.no. CK115)

Suspect or positive samples

Additional alternative testing should be performed on any suspect or positive samples in order to obtain a confirmed positive diagnosis of Mycoplasma gallisepticum or Mycoplasma synoviae within a chicken flock.

BioChek has available a software programme which can be used with the Mg/Ms kit to calculate S/P values, titres and provide general flock profiling.

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BioChek Poultry Immunoassays

TRIPLE TEST

Mycoplasma gallisepticum/synoviae & EDS Combined Antibody Test Kit

Catalogue Code CK 319

Description of Test

The Mg/Ms/EDS ELISA kit will measure the amount of antibody to Mg or Ms in the serum of chickens. Microtitre plates have been pre-coated with inactivated Mg, Ms and EDS antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-Mg, anti-Ms or anti-EDS antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti-Mg, anti-Ms or anti-EDS antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti-Mg, anti-Ms or anti-EDS antibody is present and the intensity is directly related to the amount of anti-Mg, anti-Ms or anti-EDS present in the sample.

Reagents provided

1. **Mg/Ms/EDS Coated plates.** Inactivated mycoplasma antigens and EDS viral antigen on microtitre wells.
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to Mg, Ms and EDS in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLET WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22-27°C) before use.

Sample preparation

Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove Mg/Ms/EDS coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and the samples by reading at 405 nm

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.2.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The Mg/Ms/EDS positive control has been carefully standardised to represent significant amounts of antibody to Mg or Ms in Chicken serum.

The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .5 or greater contain anti-Mg/Ms/EDS antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

Because the Mg/Ms/EDS combined test is a screening assay, it is not possible to generate antibody titre values that are valid for Mg, Ms or EDS.

The S/P generated for a sample will be for Mg, Ms or EDS. There is no differentiation between the three by this test.

For identification of a positive sample, it will be necessary to run the sample again using a monospecific Mg kit(cat. No. CK 114) or Ms(cat.no. CK115) or EDS (cat.no. CK112)

Suspect or positive samples

Additional conventional serological testing should be performed on any suspect or positive samples in order to obtain a confirmed positive diagnosis of Mycoplasma gallisepticum or Mycoplasma synoviae or Egg Drop Syndrome within a chicken flock.

BioChek has available a software programme which can be used with the Mg/Ms/EDS kit to calculate S/P values, titres and provide general flock profiling.

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BioChek Poultry Immunoassays

Salmonella enteritidis Antibody Test Kit

Detects antibodies to invasive group D salmonella species

Catalogue Code CK117

Description of Test

The S.e. ELISA kit will measure the amount of antibody to S.e. and other group D salmonella species in the serum of chickens. Microtitre plates have been pre-coated with inactivated S.e. antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti- S.e. antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti- S.e. antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti- S.e. antibody is present and the intensity is directly related to the amount of anti- S.e. present in the sample.

Reagents provided

1. **S.e. Coated plates.** Inactivated viral antigen on microtitre plates
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to S.e. in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLETS WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22-27°C) before use.

Sample preparation

Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove S.e. coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and the samples by reading at 405 nm

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.15.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The S.e. positive control has been carefully standardised to represent significant amounts of antibody to S.e. in Chicken serum.

The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .5 or greater contain anti- S.e. antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

2. Calculation of Antibody Titre

The following equation relates the S/P of a samples at a 1 : 500 dilution to an end point titre

$$\text{Log}_{10} \text{Titre} = 1.13 * \text{Log}(\text{SP}) + 3.156$$

$$\text{Antilog} = \text{Titre}$$

S/P value	Titre Range	Antibody status
.349 or less	436 or less	Negative
.350 - 0.499	437 -653	Suspect
.500 or greater	654 or greater	Positive

Additional alternative testing should be performed on any suspect or positive samples in order to obtain a confirmed positive diagnosis of *Salmonella enteritidis* within a chicken flock

BioChek has available a software programme which can be used with the S.e. kit to calculate S/P values, titres and provide general flock profiling.

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BioChek Poultry Immunoassays

Salmonella typhimurium Antibody Test Kit

Detects antibodies to invasive group B salmonella species

Catalogue Code CK118

Description of Test

The S.t. ELISA kit will measure the amount of antibody to S.t. and other group B salmonella species in the serum of chickens. Microtitre plates have been pre-coated with inactivated S.t. antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti- S.t. antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti- S.t. antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti- S.t. antibody is present and the intensity is directly related to the amount of anti- S.t. present in the sample.

Reagents provided

1. **S.t. Coated plates.** Inactivated Ips antigen on microtitre wells.
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to S.t. in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLETS WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22-27°C) before use.

Sample preparation

Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove S.t. coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and the samples by reading at 405 nm

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.2.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The S.t. positive control has been carefully standardised to represent significant amounts of antibody to S.t. in Chicken serum.

The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .5 or greater contain anti- S.t. antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

2. Calculation of Antibody Titre

The following equation relates the S/P of a samples at a 1 : 500 dilution to an end point titre

$$\text{Log}_{10} \text{Titre} = 1.13 * \text{Log}(\text{SP}) + 3.156$$

$$\text{Antilog} = \text{Titre}$$

S/P value	Titre Range	Antibody status
.349 or less	436 or less	Negative
.350 - 0.499	437 -653	Suspect
.500 or greater	654 or greater	Positive

Additional alternative testing should be performed on any suspect or positive samples in order to obtain a confirmed positive diagnosis of *Salmonella typhimurium* within a chicken flock

BioChek has available a software programme which can be used with the S.t. kit to calculate S/P values, titres and provide general flock profiling.

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BioChek Poultry Immunoassays

Salmonella enteritidis Salmonella typhimurium (combined) test Kit Detects antibodies to invasive group B and D salmonella species

Catalogue Code CK 218

Description of Test

The Se/St ELISA kit will measure the amount of antibody to Se or St and other invasive group B and D salmonella species in the serum of chickens. Microtitre plates have been pre-coated with inactivated Se and St antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-Se or anti-St antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti-Se or anti-St antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti-Se or anti-St antibody is present and the intensity is directly related to the amount of anti-Se or anti-St present in the sample.

Reagents provided

1. **Se/St Coated plates.** Inactivated Ips antigens on microtitre wells.
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to Se and St in Phosphate Buffer with protein stabilisers and sodium azide preservative(0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLET WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22-27°C) before use.

Sample preparation

Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove Se/St coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and the samples by reading at 405 nm

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.2.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The Se/St positive control has been carefully standardised to represent significant amounts of antibody to Se or St in Chicken serum.

The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .5 or greater contain anti-Se or anti-St antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

Samples with an S/P of 0.349 or less are considered NEGATIVE. Samples with an S/P of between 0.350 and .499 are considered as SUSPECT and should be re-tested

Because the Se/St combined test is a screening assay, it is not possible to generate antibody titre values that are valid for Se or St.

The S/P generated for a sample will be for Se or St. There is no differentiation between the two by this test.

For a general titre value, the following equation may be used:

$$\text{Log}_{10} \text{Titre} = 1.13 * \text{Log}(\text{SP}) + 3.156$$

$$\text{Antilog} = \text{Titre}$$

For identification of a positive sample, it will be necessary to run the sample again using a monospecific Se kit (cat. No. CK 117) or St kit (cat.no. CK118)

Suspect or positive samples

Additional alternative testing should be performed on any suspect or positive samples in order to obtain a confirmed positive diagnosis of Salmonella enteritidis or Salmonella typhimurium within a chicken flock.

BioChek has available a software programme which can be used with the Se/St kit to calculate S/P values, titres and provide general flock profiling.

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BioChek Poultry Immunoassays

Ornithobacterium rhinotracheale Antibody Test Kit For the detection of antibody to O.r. in Chickens and Turkeys

Catalogue Code CK108

Description of Test

The O.r. ELISA kit will measure the amount of antibody to O.r. in the serum of chickens and Turkeys. Microtitre plates have been pre-coated with inactivated O.r. antigen. Serum samples are diluted and added to the microtitre wells where any anti- O.r. antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti- O.r. antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti- O.r. antibody is present and the intensity is directly related to the amount of anti- O.r. present in the sample.

Reagents provided

1. **O.r. Coated plates.** O.r antigens on microtitre wells
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to O.r. in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLETS WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature before use.

Sample preparation

Dilute each test sample **1 : 100** by adding 5 µl to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove O.r. coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **60 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **60 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and the samples by reading at 405 nm

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.15.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The O.r. positive control has been carefully standardised to represent significant amounts of antibody to O.r. in Chicken or Turkey serum.

The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of 1.0 or greater contain anti- O.r. antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

2. Calculation of Antibody Titre

The following equation relates the S/P of a samples at a 1 : 100 dilution to an end point titre

$$\text{Log}_{10} \text{Titre} = 1.75 (\log_{10} \text{S/P}) + 3.156$$

$$\text{Antilog} = \text{Titre}$$

S/P value	Titre Range	Antibody status
.499 or less	424 or less	Negative
.500 - .999	425 - 1431	Suspect
1.0 or greater	1432 or greater	Positive

Each Laboratory should establish its own criteria for non protected and protected

BioChek has available a software programme which can be used with the O.r. kit to calculate S/P values, titres and provide general flock profiling.

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BioChek Poultry Immunoassays

Avian Leukosis Virus (p27) Test Kit

Catalogue Code CK111

Description of Test

The ALV-Ag (p27) ELISA kit will measure the amount of antigen in various different samples taken from chickens. Microtitre plates have been pre-coated with anti-p27 antibody. Test samples are added to the microtitre wells where any p27 antigen present will bind and form an antigen-antibody complex. Non specific antigens and other sample proteins are then washed away. Anti-p27 antibody labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any p27 antigen originally bound to antibody. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if p27 antigen is present and the intensity is directly related to the amount of p27 antigen present in the sample.

Reagents provided

1. **Anti-p27 Coated plates.** Anti-p27 antibody on microtitre wells.
2. **Conjugate reagent.** Rabbit anti-p27: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Wash Buffer concentrate.** Phosphate Buffered Saline with Tween and sodium azide preservative (1% w/v) (x10 conc.)
7. **Negative control.** Phosphate Buffer with protein stabilisers, inert blue dye and sodium azide preservative (0.1% w/v)
8. **Positive Control.** Inactivated p27 antigen in Phosphate Buffer with protein stabilisers, inert red dye and sodium azide preservative(0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLETS WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or de-ionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22-27°C) before use.

Sample preparation

1. **Thin Albumen** samples are run neat in the test (no dilution of sample required)
2. **Swab or meconia** samples must be taken into 1 ml. of PBS buffer and shaken well before testing (freezing and thawing can expose more antigen).
3. A fresh pipette tip must be used for each separate sample.

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove ANTI-p27 coated plate from sealed bag and record location of samples on template.
2. Add 50 µl of samples to microtitre wells
3. Add 50 µl of negative control into wells A12 and B12
4. Add 50 µl of positive control into wells C12 and D12
5. Cover plate with lid and incubate at room temperature (22-27°C) **for 30 minutes**
6. Aspirate contents of wells and wash 5 times with wash buffer (400µl per well). Invert plate and tap firmly on absorbent paper.
7. Add 50 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) **for 30 minutes**.
8. Repeat wash procedure as in 5.
9. Add 50 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) **for 20 minutes**.
10. Add 50 µl of Stop Solution to appropriate wells to stop reaction.
11. Blank the microtitre plate reader on air and record the absorbance of controls and the samples by reading at 405 nm.

Results:

For the test result to be valid the mean negative control absorbance should read **below 0.15** and the difference between the mean negative control and the mean positive control should be **greater than 0.5**.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The p27 positive control has been carefully standardised to represent significant amounts of p27 ANTIGEN found in Chicken test samples.

The relative amounts of p27 in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

Albumen

Samples with an S/P of .2 or greater contain p27 antigen and are considered POSITIVE.

Samples with an S/P below 0.1 are considered NEGATIVE(between 0.101 and .199 samples are considered BORDERLINE)

Other samples than Albumen - (Swab, meconia etc.)

Samples with an S/P of .3 or greater contain anti- p27 antigen and are considered POSITIVE.

Samples with an S/P below 0.2 are considered NEGATIVE(between 0.201 and .299 samples are considered BORDERLINE)

BioChek has available a software programme which can be used with the ALV -ANTIGEN (p27) TEST kit to calculate S/P values and provide general flock profiling.

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BioChek Poultry Immunoassays

Avian Encephalomyelitis Antibody Test Kit

Catalogue Code CK123

Description of Test

The AE ELISA kit will measure the amount of antibody to AE in the serum of chickens. Microtitre plates have been pre-coated with inactivated AE antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-AE antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti-AE antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti-AE antibody is present and the intensity is directly related to the amount of anti-AE present in the sample.

Reagents provided

1. **AE Coated plates.** Inactivated viral antigen on microtitre plates
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to AE in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLETS WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22 - 27 °C) before use.

Sample preparation

Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove AE coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and samples by reading at 405 nm.

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.2.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The AE positive control has been carefully standardised to represent significant amounts of antibody to AE in Chicken serum.

The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .5 or greater contain anti-AE antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

2. Calculation of Antibody Titre

The following equation relates the S/P of a samples at a 1 : 500 dilution to an end point titre

$$\text{Log}_{10} \text{Titre} = 1.1 * \text{Log}(\text{SP}) + 3.361$$

$$\text{Antilog} = \text{Titre}$$

S/P value	Titre Range	Antibody status
.349 or less	722 or less	Negative
.350 - 0.499	723 - 1070	Suspect
.500 or greater	1070 or greater	Positive

Each Laboratory should establish its own criteria for non protected and protected

BioChek has available a software programme which can be used with the AE kit to calculate S/P values, titres and provide general flock profiling.

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BioChek Poultry Immunoassays

Avian Influenza Antibody Test Kit

Catalogue Code CK 121

Description of Test

The AI ELISA kit will measure the amount of antibody to AI in the serum of chickens and Turkeys. Microtitre plates have been pre-coated with inactivated AI antigen. Serum samples are diluted and added to the microtitre wells where any anti-AI antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti-AI antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti-AI antibody is present and the intensity is directly related to the amount of anti-AI present in the sample.

Reagents provided

1. **AI Coated plates.** Inactivated viral antigen on microtitre plates
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to AI in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLETS WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22 - 27 °C) before use.

Sample preparation

Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove AI coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and samples by reading at 405 nm.

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.3.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The AI positive control has been carefully standardised to represent significant amounts of antibody to AI in chicken or turkey serum.

The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .5 or greater contain anti-AI antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

2. Calculation of Antibody Titre

The following equation relates the S/P of a samples at a 1 : 500 dilution to an end point titre

$$\text{Log}_{10} \text{Titre} = 1.1 * \text{Log}(\text{SP}) + 3.156$$

$$\text{Antilog} = \text{Titre}$$

S/P value	Titre Range	Antibody status
.499 or less	667 or less	Negative
.500 or greater	668 or greater	Positive

Additional alternative testing should be performed on any suspect or positive samples in order to obtain a confirmed positive diagnosis of Avian Influenza within a chicken or Turkey flock.

BioChek has available a software programme which can be used with the AI kit to calculate S/P values, titres and provide general flock profiling.

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BioChek Poultry Immunoassays

Infectious Laryngotracheitis Antibody Test Kit (STRIP PLATE FORMAT)

Catalogue Code CK213

Description of Test

The ILT ELISA kit will measure the amount of antibody to ILT in the serum of chickens. Microtitre plates have been pre-coated with inactivated ILT antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-ILT antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti-ILT antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow color is developed if anti-ILT antibody is present and the intensity is directly related to the amount of anti-ILT present in the sample.

Reagents provided

1. **ILT Coated plates.** Inactivated viral antigen on microtitre plates
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to ILT in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or de-ionized water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially bio hazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidizing agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for *IN VITRO* use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C.*

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLETS WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or de-ionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22 - 27 °C) before use.

Sample preparation

Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove ILT coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **60 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **60 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and samples by reading at 405 nm.

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.15.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The ILT positive control has been carefully standardized to represent significant amounts of antibody to ILT in Chicken serum.

The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .5 or greater contain anti-ILT antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

2. Calculation of Antibody Titre

The following equation relates the S/P of a samples at a 1 : 500 dilution to an end point titre

$$\text{Log}_{10} \text{Titre} = 1.1 * \text{Log}(\text{SP}) + 3.361$$

$$\text{Antilog} = \text{Titre}$$

S/P value	Titre Range	Antibody status
.500 or less	1070 or less	Negative
.501 or greater	1071 or greater	Positive

Each Laboratory should establish its own criteria for non protected and protected

BioChek has available a software program which can be used with the ILT kit to calculate S/P values, titres and provide general flock profiling.

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BioChek Poultry Immunoassays

Reticuloendotheliosis Antibody Test Kit (STRIP PLATE FORMAT)

Catalogue Code CK 125

Description of Test

The REV ELISA kit will measure the amount of antibody to REV in the serum of chickens. Microtitre plates have been pre-coated with inactivated REV antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-REV antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti-REV antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow color is developed if anti-REV antibody is present and the intensity is directly related to the amount of anti-REV present in the sample.

Reagents provided

1. **REV Coated plates.** Inactivated viral antigen on microtitre plates
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets.** PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer.** Diethanolamine buffer with enzyme co-factors
5. **Stop Solution.** Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent.** Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer.** Powdered Phosphate Buffered Saline with Tween
8. **Negative control.** Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to REV in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or de-ionized water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially bio hazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidizing agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for *IN VITRO* use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent.** To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 °C*.

Drop tablets into clean container and add appropriate volume of Substrate Buffer

DO NOT HANDLE TABLETS WITH BARE FINGERS

2. **Wash Buffer.** Empty the contents of one wash buffer sachet into one litre of distilled or de-ionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
3. All other kit components are ready to use but allow to come to room temperature (22 - 27 °C) before use.

Sample preparation

Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:

1. Remove REV coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and samples by reading at 405 nm.

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.2.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The REV positive control has been carefully standardized to represent significant amounts of antibody to REV in Chicken serum.

The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .2 or greater contain anti-REV antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

$$\frac{\text{Mean of Test Sample} - \text{Mean of negative control}}{\text{Mean of Positive control} - \text{Mean of negative control}} = \text{S/P}$$

2. Calculation of Antibody Titre

The following equation relates the S/P of a samples at a 1 : 500 dilution to an end point titre

$$\text{Log}_{10} \text{Titre} = 1.1 * \text{Log}(\text{SP}) + 3.361$$

$$\text{Antilog} = \text{Titre}$$

S/P value	Titre Range	Antibody status
.500 or less	1070 or less	Negative
.501 or greater	1071 or greater	Positive

Each Laboratory should establish its own criteria for non protected and protected

BioChek has available a software program which can be used with the REV kit to calculate S/P values, titres and provide general flock profiling.

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