English

ProFLOK™ AIV BE Ab

zoetis

Zoetis Inc.
Kalamazoo, MI 49007, USA
VLN/PCN 190/5004.21
www.zoetis.com

For product info call:
US VMIPS 1-888-963-8471
Canada 1-800-461-0917

EC REP

ZOETIS FRANCE
23 Rue Pierre Gilles de Gennes,
69007 Lyon, FRANCE

40018905
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AVIAN INFLUENZA VIRUS ANTIBODY TEST KIT, cELISA

For the detection of antibodies to Avian Influenza Virus (AIV) in chicken and turkey sera.

GENERAL INFORMATION AND INTENDED USES

ProFLOK AIV BE is an in-vitro blocking ELISA designed to aid in the qualitative detection of AIV antibodies in chickens and turkeys. The assay detects all 16 subtypes of AIV. Positive results should be submitted to a reference lab for confirmation and subtype determination. Negative results indicate that no detectable AIV antibody is present.

KIT COMPOSITION AND CONSERVATION

Contains materials sufficient to test a maximum of 450 samples.

ITEM	REAGENT NATURE	VOLUME	RECONSTITUTION AND CONSERVATION
A	5 microplates containing 96 wells coated with AIV antigen	5 X 96 wells	Ready to use
CONTROL +	BE Positive Control; preserved with Microcide III	1 X 1.4 mL	Ready to use
CONTROL -	BE Negative Control; preserved with Microcide III	1 X 1.4 mL	Ready to use
C	100X BE HRP-Conjugate; preserved with Microcide III	1 X 0.9 mL	Dilute in Dilution Buffer Plus just before use.
DB	Dilution Buffer; preserved with Microcide III	1 X 100 mL	Ready to use
W	20X Wash; preserved with Imidazole	1 X 100 mL	Dilute to 1X in deionized or reverse osmosis water. Diluted wash solution can be stored at 15 °C - 30 °C and used for up to 3 months following dilution.
ABTS	Substrate	1 X 100 mL	Ready to use
S	5X Stop (5 % SDS)	1 X 25 mL	Dilute to 1X in deionized or reverse osmosis water. Diluted stop solution can be stored at 15 °C - 30 °C and used for up to 3 months following dilution.

Store all reagents provided in the kit at 2 – 7 °C. Reagents should not be frozen.

REAGENTS REQUIRED TO PERFORM 90 TESTS

a) 1 AIV antigen coated microplate
b) 200 µL BE Positive Control
c) 200 µL BE Normal Control
d) 110 µL 100X Conjugate
e) 20 mL Dilution Buffer
f) 20 mL 20X Wash
g) 10 mL Substrate
h) 2.5 mL 5X Stop

EQUIPMENT AND MATERIALS REQUIRED, BUT NOT PROVIDED

a) High precision multiple delivery pipetting devices (i.e., 1-20 and 20-200 µL. Measurement deviation must be ≤10 % for volumes ≤10 µL and ≤ 5 % for all other volumes)
b) 8- or 12-channel pipettes (i.e., 5-50 and 50-300 µL) and pipette tips
c) 0.2 mL, 1.0 mL, and 5.0 mL pipettes
d) 2-3 graduated cylinders (50 mL)
e) 1 mL or 5 mL glass test tubes
f) Uncoated low binding 96 well microplates with > 300 µL/well volume
g) Deionized or reverse osmosis water
h) Microplate reader with 405-410 nm filter
i) Microplate washing apparatus

WARNINGS TO THE USERS OF REAGENTS AND ANTIGEN COATED MICROPLATES

- Handle all reagents and samples as biohazardous material. It is recommended to dispose reagents and contaminated material according to the applicable regulations.
- Wear suitable protective clothing.
- Irritating to eyes and skin. Keep all reagents away from eyes and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Take care not to contaminate any test reagents with serum or bacterial agents.
- If the humidity indicator of a microplate exhibits a pink color, the microplate should not be used.
- The best results are achieved by following the protocols described below, using good, safe laboratory techniques.
- Never add water to the microplates, conjugate, controls, or substrate.
- Do not use this kit after the expiration date.
- NEVER PIPETTE BY MOUTH. Harmful if swallowed.
- For animal use only.

Refer to the end of this insert for reagent hazard and precaution statements. Also reference the Safety Data Sheet for additional details.

SAMPLE COLLECTION

For routine serologic flock monitoring:

- Randomly collect a statistically significant number of samples at routine intervals (for example, collect 30 sera every 21 days).
- Follow proper sample collection procedures.
- Harvest serum and store properly (up to seven days at 4 °C, -20 °C for longer).
- Test only good quality serum (i.e., avoid bacterial contamination, heavy hemolysis or lipemia). When in doubt, obtain a better quality sample.

Allow all reagents to come to 21 – 24 °C before starting.

PREPARATION OF 1X POSITIVE CONTROL, 1X CONJUGATE, 1X WASH, AND 1X STOP SOLUTIONS

STEP	UNITS	MATERIAL	LOCATION	NOTES
1X CONJUGATE SOLUTION				
1)	10.9 mL	Dilution Buffer	Clean tube or bottle	Mix well. 1:100 final dilution.
2)	110 µL	100X BE Conjugate		
1X WASH SOLUTION				
3)	20 mL	20X Wash	Microplate washing bottle or apparatus	Mix well. 1:20 final dilution.
4)	380 mL	Deionized or reverse osmosis water		
1X STOP SOLUTION				
5)	2.5 mL	5X Stop	Clean tube or bottle	Warm 5X Stop to 21-24 °C or to 37 °C and mix to dissolve any precipitates.
6)	10 mL	Deionized or reverse osmosis water		Mix well. 1:5 final dilution

ELISA TEST PROCEDURE

STEP	UNITS	MATERIAL	LOCATION	NOTES
a)	Remove the test plate from the protective bag. If using a plate with removable wells, break off the required number of wells to perform the assay and place them in the well holder. For each assay, two wells are required for the BE Positive Control and two wells are required for the BE Negative Control. Each sample may be tested in a single well. Seal any unused coated wells in the desiccated mylar pouch for future use and return immediately to 2 to 7°C storage.			
b)	75 µL	BE Dilution Buffer	Add into each test microplate well	
c)	25 µL	Serum sample	Add into each test microplate well	1:4 final dilution. Discard pipette tips.
d)	100 µL	BE Positive Control	Designated wells	Discard pipette tips.
e)	100 µL	BE Negative Control	Designated Wells	Discard pipette tips.
f)	Gently tap the side of the plate to mix contents and incubate for 60 minutes at 21 – 24 °C.			
g)	At the end of the incubation period, discard the fluid from the wells into an appropriate vessel containing bleach or other decontamination agent.			

ADDITION OF CONJUGATE

h)	100 µL	1X BE Conjugate Solution (step 9)	Each test well	Discard pipette tips.
i)	Incubate for 30 minutes at 21 – 24 °C.			

WASH PROCEDURE

j)	Discard or aspirate solution from all wells.			
k)	350 µL	1X Wash Solution (step 11)	Each test well	**Wash process is a critical step for an ELISA. Please follow steps j to m.**
l)	Discard or aspirate solution from all wells.			
m)	**Repeat wash procedure 4 more times for a total of 5 washes**			
n)	After plate wash is complete, tap the inverted plate onto paper towels to ensure that all residual liquid is removed.			

ADDITION OF SUBSTRATE, AND 1X STOP SOLUTION

o)	100 µL	Substrate	Each test well	Discard pipette tips.
p)	Incubate for 15 minutes at 21 – 24 °C.			
q)	100 µL	1X Stop Solution (step 13)	Each test well	Discard pipette tips.
r)	Read the microplate using an ELISA microplate reader set at 405–410 nm. Be sure to blank the reader as directed. Allow bubbles to dissipate and wipe the bottom of the microplate before reading.			

RESULTS

a) Calculate the mean optical density (OD) of the BE Negative Control wells.
b) To determine the Sample to Negative (S/N) ratio of a sample, divide the Sample OD by the Mean OD of the BE Negative Control:

$$\text{S/N} = \frac{\text{SAMPLE OD}}{\text{MEAN BE NEGATIVE CONTROL OD}}$$

c) Samples with an S/N ratio of **less than 0.60** are considered **POSITIVE** for antibody against Avian Influenza
d) Samples with an S/N ratio of **greater than or equal to 0.60** are considered **NEGATIVE** for antibody against Avian Influenza.

Assay Control Values, Valid ELISA Results

Valid ELISA results are obtained when the mean optical density (OD) of the BE Negative Control is 0.300 to 0.700, and the mean OD of the BE Positive Control is < 0.250. If either of these values is out of range, the test results should be considered invalid and the samples should be retested.

SYMBOL DESCRIPTIONS

Authorized Representative in the European Community

Temperature limitations (storage temperature range)

Use by (expiration date)

In vitro diagnostic medical device

Batch Code

Manufacturer

Serial Number

Date of manufacture

Postive control

Consult instructions for use

Negative control

WARNING

H319 / P264 / P280 / P305 + P351 + P338 / P337 + P313

Wash solution – Causes serious eye irritation. Wash thoroughly after handling. Wear protective gloves/protective clothing/eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention. May damage the unborn child. Do not handle until all safety precautions have been read and understood. If exposed or concerned: get medical advice/attention.

H412 / P273 / P501

Stop solution – Store away from incompatible materials. Dispose of contents/container in accordance with local/regional/national/international regulations.

DANGER

H360FD / P201 / P202 / P280 / P308 + P313 / P405 / P501

Dilution Buffer – May cause cancer. May damage fertility. May damage the unborn child. Obtain special instructions before use. Do not handle until all safety precautions have been read and understood. Wear protective gloves/protective clothing/eye protection/face protection. If exposed or concerned: get medical advice/attention. Store locked up. Dispose of contents/container in accordance with local/regional/national/international regulations.