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ProFLOK™ ALV Ag

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AVIAN LEUKOSIS VIRUS ANTIGEN TEST KIT
For the detection of Avian Leukosis Virus (ALV) p27 antigen in chicken serum and egg albumin.

GENERAL INFORMATION AND INTENDED USES
ProFLOK™ ALV Ag is a rapid screening ELISA for the detection of ALV p27 antigen in chickens.

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 ALV p27 antibody	5 X 96 wells	Ready to use
CONTROL +	Positive Control	1 X 2.0 mL	Ready to use
CONTROL -	Negative Control	1 X 2.0 mL	Ready to use
C	50X HRP-Conjugate; preserved with Microcide III	1 X 1.3 mL	Dilute in Dilution Buffer just before use.
DB	Dilution Buffer	1 X 200 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 - 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 - 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 ALV anti-p27 antibody coated microplate
b) 300 µL Positive Control
c) 300 µL Negative Control
d) 200 µL 50X Conjugate
e) 10 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 graduated cylinders (50 mL)
e) 1 mL or 5 mL test tubes
f) Uncoated low binding 96 well microplates
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.

SAMPLE DILUTION PROCEDURE

Dilute serum samples using the dilution buffer provided in a clean, uncoated 96 well microplate (Sample Dilution Microplate). Samples should be completely thawed and thoroughly mixed before diluting. **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 mL	Dilution Buffer	Clean tube or bottle	Mix well. 1:50 final dilution.
2)	200 µL	50X 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 microplate from protective bag and label the microplate with the flock/sample positions as in step 2.			
b)	100 µL	Negative Control	Into wells A2, H10, and H12	Do not dilute. Discard pipette tips.
c)	100 µL	Positive Control	A1, A3, and H11	Do not dilute. Discard pipette tips.
d)	100 µL or 2 drops	Sample serum or Egg albumin	Into wells A4-H9, left to right, row by row	Discard pipette tips.
	Incubate for 30 minutes at 21 – 24 °C.			

WASH PROCEDURE

STEP	UNITS	MATERIAL	LOCATION	NOTES
f)	Discard or aspirate solution from all wells.			Tap inverted plate.
g)	300 µL	1X Wash Solution (step 11)	Each test well	Soak for 3 minutes
h)	After 3 minute soak, aspirate all wells; tap inverted plate to remove residual liquid.			**Wash process is a critical step for an ELISA. Please follow steps f to i.**
i)	**Repeat wash procedure 2 more times.**			

ADDITION OF 1X CONJUGATE, SUBSTRATE, AND 1X STOP SOLUTION

STEP	UNITS	MATERIAL	LOCATION	NOTES
j)	100 µL	1X Conjugate Solution (step 2)	Each test well	Discard pipette tips.
k)	Incubate for 30 minutes at 21 – 24 °C.			
l)	Follow the **WASH PROCEDURE** above (steps f to i).			
m)	100 µL	Substrate	Each test well	Discard pipette tips.
n)	Incubate for 15 minutes at 21 – 24 °C.			
o)	100 µL	1X Stop Solution (step 6)	Each test well	Discard pipette tips.
p)	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

ASSAY CONTROL VALUES, VALID ELISA RESULTS

Valid ELISA results are obtained when the Negative Control Average optical density (OD) is < 0.250 and the Corrected Positive Control (CPC) is between 0.150 and 1.200. If either of these values is out of range, the test results should be considered invalid and the samples should be retested.

MANUAL PROCESSING OF DATA

a) Average the OD values of Positive Control in wells A1, A3, and H11 then average the OD values of Negative Control in wells A2, H10, and H12. Record both averages.
b) Subtract the average Negative Control OD from the average Positive Control OD. The difference is the Corrected Positive Control.
c) Calculate a sample to positive (S/P) ratio by subtracting the average Negative Control OD from each sample OD and dividing the difference by the Corrected Positive Control. Use the following equation format:

$$S/P = \frac{(\text{SAMPLE OD}) - (\text{AVERAGE NEGATIVE CONTROL OD})}{\text{CORRECTED POSITIVE CONTROL}}$$

INTERPRETATION OF RESULTS

The ALV ELISA titer values obtained represent a comparisons of the unknown antigen level of the sample to the ALV ELISA kit positive control antigen. Therefore, it is important to first determine that the ALV ELISA positive and negative control values obtained are valid as detailed above in the "Assay Control Values, Valid ELISA Results" section of this pamphlet before ALV ELISA results are interpreted.

The ALV S/P values obtained for sera or albumin should be interpreted as follows:

ALV Presumed Antigen Status:

a. **Negative.** Samples testing with an ALV S/P value of < 0.199 will receive a "0" titer value and are presumed negative for p-27 antigen. A "0" ALV ELISA titer represents a chicken serum or albumin sample that contains an extremely low to insignificant ALV p27 antigen level compared to the ALV ELISA kit positive and negative controls.
b. **Positive.** An ALV ELISA titer value above "0" indicates only that a chicken serum or albumin sample contains a significant and ELISA-detectable ALV p27 antigen level compared to the ALV ELISA kit positive and negative control sera.