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ProFLOK™ REO Ab

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AVIAN REOVIRUS ANTIBODY TEST KIT

For the detection of antibodies to Avian Reovirus (REO) in chicken serum.

GENERAL INFORMATION AND INTENDED USES

ProFLOK™ REO Ab is a rapid screening ELISA for the detection of pre- and post-vaccination REO antibodies 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 REO antigen	5 X 96 wells	Ready to use
CONTROL+	100X Positive Control; preserved with Thimerosal	1 X 0.1 mL	Dilute in Dilution Buffer just before use.
N	100X Normal Control; preserved with Thimerosal	1 X 0.1 mL	Dilute in Dilution Buffer just before use.
C	100X HRP-Conjugate; preserved with Microcide III	1 X 1.0 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 REO antigen coated microplate
b) 10 µL 100X Positive Control
c) 10 µL 100X Normal Control
d) 120 µL 100X Conjugate
e) 46 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 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.

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.**

STEP	UNITS	MATERIAL	LOCATION	FINAL DILUTION	NOTES
1)	300 µL	Dilution Buffer	Each well	N/A	
2)	6 µL	Sample Serum	Add into wells A4 - H9; left to right, row by row	1:50	Mix. Discard tips after each sample. Label the microplate to identify the flock/ sample positions.
3)	6 µL	100X Normal Control	Into wells A2, H10, and H12	1:50	
4)	Aspirate wells A1, A3, and H11.				
5)	Allow all diluted sera to equilibrate for 5 minutes before transferring to the ELISA microplate.				

Note: This sample dilution microplate provides adequate quantities of diluted serum samples to conduct four additional ProFLOK™ ELISA tests. Use dilution microplate within 24 hours.

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

STEP	UNITS	MATERIAL	LOCATION	NOTES
1X POSITIVE CONTROL SOLUTION				
6)	300 µL	Dilution buffer	Clean test tube	Mix well. 1:50 final dilution.
7)	6 µL	100X Positive Control		
1X CONJUGATE SOLUTION				
8)	12 mL	Dilution buffer	Clean tube or bottle	Mix well. 1:100 final dilution.
9)	120 µL	100X Conjugate		
1X WASH SOLUTION				
10)	20 mL	20X Wash	Microplate washing bottle or apparatus	Mix well. 1:20 final dilution.
11)	380 mL	Deionized or reverse osmosis water		
1X STOP SOLUTION				
12)	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.
13)	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)	50 µL	Dilution buffer	Add into each test microplate well	
c)	50 µL	1X Positive Control Solution (step 7)	A1, A3, and H11	Discard pipette tips. 1:100 final dilution.
d)	50 µL	Sample Dilution Microplate (step 5)	Transfer to the matching wells of the test microplate	Quickly transfer each row. Discard pipette tips. 1:100 final dilution.
e)	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 9)	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 13)	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 Normal Control Average optical density (OD) is < 0.250 and the Corrected Positive Control (CPC) is between 0.250 and 0.900. If any of these values are 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 Normal Control in wells A2, H10, and H12. Record both averages.
b) Subtract the average Normal 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 Normal Control OD from each sample OD and dividing the difference by the Corrected Positive Control. Use the following equation format:

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

d) An ELISA titer for REO can be calculated by the following suggested equation:
LOG_{10} TITER = (1.077 X LOG_{10} S/P) + 3.460
TITER = ANTILOG of LOG_{10} TITER

EXAMPLE:

Example Positive Control ODs:
0.585, 0.610, 0.590
Average = (0.585 + 0.610 + 0.590) / 3 = 0.595

Corrected Positive Control:
(0.595) – (0.067) = 0.528

Example Normal Control ODs:
0.078, 0.067, 0.057
Average = (0.078 + 0.067 + 0.057) / 3 = 0.067

Example S/P value calculation:
OD of sample = 0.560
(0.560) – (0.067) / 0.528 = 0.934

Example of Calculation of titer:
LOG_{10} Titer = (1.077 X LOG_{10} 0.934) + 3.460
Titer = ANTILOG 3.43
Titer = 2680

INTERPRETATION OF RESULTS

The REO ELISA titer values obtained represent a comparison of the REO antibody level within each field chicken serum tested and the REO ELISA kit positive and normal control sera. Therefore, it is important to first determine that the REO ELISA positive and normal control sera values obtained are valid as detailed above in the "Assay Control Values, Valid ELISA Results" section of this pamphlet before REO ELISA results are interpreted.

The REO S/P values obtained for sera should be interpreted as follows:

REO Presumed Antibody Status:

a. **Negative.** Serum samples with a REO S/P ratio value of ≤ 0.150 receive a "0" titer value and are presumed negative for REO antibody. A "0" REO ELISA titer represents a chicken serum sample that contains an extremely low to insignificant REO antibody level compared to the REO ELISA kit positive and normal control sera.
b. **Positive.** A REO ELISA titer value above "0" indicates only that a chicken serum sample contains a significant and ELISA-detectable REO antibody level compared to the REO ELISA kit positive and normal control sera. However, these titers do **not** imply or ensure "protection" nor provide serologic differentiation between a REO vaccine response or REO field infection.

Optimal REO vaccine administration practices and "protective" flock REO titer target values must be determined by each REO ELISA kit user by comparing flock pre- and post-vaccination REO ELISA results (i.e., coefficient of variation [%CV] and geometric titer [GMT] values) with flock performance parameters, (i.e., morbidity, mortality, flock body weight gain or uniformity) over time.