MYCOPLASMA GALLISEPTICUM-SYNOVIAE ANTIBODY TEST KIT

ITEM NO. 96-6531

ProFLOK®

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U.S. VET. LIC. NO. 312

MYCOPLASMA GALLISEPTICUM-SYNOVIAE ANTIBODY TEST KIT

GENERAL INFORMATION AND INTENDED USES

This ELISA kit is designed to detect antibody to *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS).

This ProFLOK® ELISA Kit is a rapid and specific presumptive screening test for the detection of antibody to MG and MS strains in chicken and turkey serum samples. It was designed for screening large numbers of chicken and turkey sera from numerous flocks; however, additional conventional MG and MS serologic testing [i.e. serum plate aggluntination (SPA) and hemagglutination-inhibition (HI) test] and culture techniques are needed to confirm MG or MS negative and MG or MS infected chicken or turkey flocks.

The assay is designed to measure antibody bound to antigen coated plates. The principle of the test is as follows: Serum obtained from chickens or turkeys exposed to MG or MS antigens contain specific MG or MS antibodies. Serum, diluted in Dilution Buffer, is added to an antigen coated plate. Specific antibody in the serum forms an antibody-antigen complex with the antigen bound to the plate. After washing the plate, an affinity purified goat anti-chicken IgG (H+L) peroxidase conjugate is added to each well. The antibody-antigen complex remaining from the previous step binds with the conjugate. After a brief incubation period, the unbound conjugate is removed by a second wash step. Substrate, which contains a chromagen (ABTS), is added to each well. Chromagen color change (from clear to green-blue) occurs in the presence of the peroxidase enzyme. The relative intensity of color developed in 15 minutes (compared to controls) is directly proportional to the level of antibody in the serum. After the substrate has incubated, Stop Solution is added to each well to terminate the reaction and the plate is read using an ELISA plate reader at 405-410 nm.

REAGENTS REQUIRED TO PERFORM 90 TESTS

a) 1 antigen coated plate (MG/MS)
b) 10 µL Positive Control Serum (MG/MS)
c) 10 µL Normal Control Serum (MG/MS)
d) 100 µL Goat anti-Chicken IgG (H+L) Peroxidase Conjugate Solution (MG/MS)
e) 40 mL Dilution Buffer Plus
f) 10 mL ABTS-Hydrogen Peroxide Substrate Solution
g) 2.5 mL 5X Stop Solution, 5% SDS (dilute [1:5] with laboratory grade water)
h) 20 mL 20X Wash Solution (dilute [1:20] with laboratory grade water)

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

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

a) High precision pipette (i.e. 1-20 microliter pipette)
b) 0.2 mL, 1.0 mL, and 5.0 mL pipettes
c) 8 or 12 channel pipette (or transplating device) and pipette tips
d) 2 graduated cylinders (50 mL)
e) 1 mL or 5 mL borosilicate glass test tubes
f) Uncoated low binding 96 well test plates (i.e. Nunc catalog #269620)
g) Laboratory grade (distilled or R.O.) water
h) 96 well plate reading spectrophotometer with 405-410 nm filter
i) Plate washing apparatus
j) Waste container with bleach or other oxidizing agent

WARNINGS TO THE USERS OF REAGENTS AND ANTIGEN COATED PLATES

a) Handle all reagents and samples as biohazardous material.
b) Keep all reagents away from skin and eyes. If exposure should occur, immediately flush affected areas with cold water.
c) Wash solution, control sera, test plates, field samples and all other test kit reagents should be properly decontaminated with bleach or other strong oxidizing agent before disposal
d) Take special care not to contaminate any of the test reagents with serum or bacterial agents.
e) Humidity indicators are supplied with each plate. If any of the indicators exhibit a pink color, the plate may be compromised in some way; decontaminate (i.e. wash the plate with bleach solution) and dispose of the plate.
f) The best results are achieved by following the protocols as they are described below, using good, safe laboratory techniques.
g) Do not use this kit after the expiration date.
h) **NEVER PIPETTE BY MOUTH.**

ALLOW ALL REAGENTS TO COME TO ROOM TEMPERATURE BEFORE STARTING!

SAMPLE COLLECTION

For routine serologic flock monitoring, it is suggested that at least **30 or more sera per flock** be randomly collected at standard time intervals (i.e. every four weeks). Proper sample collection procedures, serum harvest and serum sample storage (4°C for up to four days or -20°C for longer periods) are needed to provide reliable test results. Test only good quality serum (i.e. avoid bacterial contamination, heavy hemolysis or clotted fat).

SAMPLE DILUTION PROCEDURE

Dilute serum samples using Dilution Buffer in a clean, uncoated 96 well microtiter plate. Frozen serum samples should be completely thawed and thoroughly mixed before diluting. Set up samples and controls as shown in Figure 1.

PREPARATION OF THE SERUM DILUTION PLATE

a) Add 300 µL Dilution Buffer to each well of an uncoated 96 well microtiter plate. This plate is referred to as the serum dilution plate.

b) Add 6 µL unknown serum per well as per Figure 1 (producing a 1:50 dilution). Start with well A4 and end with well H9 (moving left to right, row by row of wells). For example, wells 1 through 30 contain the diluted sera of flock 1, wells 31 -60 contain the diluted sera of flock 2, etc.

c) Add 6 µL of Normal Control Serum (producing a 1:50 dilution) to wells A2, H10, and H12.

d) Aspirate and remove any liquid in dilution plate wells A1, A3, and H11.

e) Allow all diluted serums to equilibrate in Dilution Buffer for 5 minutes before transferring to an antigen coated ELISA plate.

f) Diluted serum should be tested within 24 hours. This dilution format provides adequate quantities of diluted serum samples to conduct three additional ProFLOK® ELISA tests using the same serum dilution plate.

Preparation of Positive Control

A Positive Control Serum for each agent has been provided with this kit. Dilute the appropriate volume of the appropriate Positive Control Serum with Dilution Buffer (1:50) in a clean, glass test tube. For example, dilute 6 µL of positive control serum in 300 µL Dilution Buffer. **Mix well.** 150 µL of diluted Positive Control is needed per ELISA plate.

Preparation of Conjugate Solution

The horseradish peroxidase conjugated anti-chicken IgG (H+L) is supplied in HRP stabilizer. Dilute 100 µL stock conjugate in 10 mL Dilution Buffer (1:100 dilution). **Mix well.** This 10 mL preparation will supply sufficient conjugate for one 96 well ELISA plate.

Preparation of 1X Wash Solution

Dilute 20 mL concentrated Wash Solution in 380 mL laboratory grade (distilled or R.O.) water (1:20). Mix well. Approximately 400 mL Wash Solution is needed for each 96 well ELISA plate.

Preparation of the Substrate Solution

The Substrate Solution is ready to use. Each plate will require approximately 10 mL substrate solution. For best results, the substrate solution must be equilibrated to room temperature before use.

Preparation of 1X Stop Solution

Dilute 2.5 mL concentrated Stop Solution in 10 mL laboratory grade (distilled or R.O.) water (1:5). **Mix well.** Approximately 12.5 mL Stop Solution is needed for each 96 well ELISA plate.

NOTE: Storage of 5X Stop Solution at refrigerated temperatures may cause the formation of a white solid. This does not affect product performance. Warm at room temperature or 37°C to dissolve before use.

Figure 1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	+	-	+	1	2	3	4	5	6	7	8	9
B	10	11	12	13	14	15	16	17	18	19	20	21
C	22	23	24	25	26	27	28	29	30	31	32	33
D	34	35	36	37	38	39	40	41	42	43	44	45
E	46	47	48	49	50	51	52	53	54	55	56	57
F	58	59	60	61	62	63	64	65	66	67	68	69
G	70	71	72	73	74	75	76	77	78	79	80	81
H	82	83	84	85	86	87	88	89	90	-	+	-

ELISA TEST PROCEDURE

PREPARING THE TEST PLATE

a) Remove an antigen coated test plate from the protective bag and label according to the dilution plate identification.
b) Add 50 µL Dilution Buffer to all wells on the test plate.
c) Add 50 µL diluted Positive Control Serum to wells A1, A3, and H11. Discard pipette tip.
d) Using an 8 or 12 channel pipette transfer 50 µL/well of each of the diluted serum samples and Normal Control Serum samples from the dilution plate to the corresponding wells of the coated test plate (yields a 1:100 dilution. Discard pipette tips after each row of sample is transfered of samples to the ELISA plate should be done as quickly as possible.
e) Incubate plate for 30 minutes at room temperature.

WASH PROCEDURE

f) Tap out liquid from each well into an appropriate vessel containing bleach or other decontamination agent.
g) Using an 8 or 12 channel pipette (or comparable automatic washing device), fill each well with approximately 300 µL Wash Solution. Allow to soak in wells for 3 minutes; then discard contents into an appropriate waste container (waste container should contain bleach solution). Tap inverted plate to ensure that all residual liquid is removed.

Repeat wash procedure 2 more times.

NOTE: The wash procedure is a very critical step in any ELISA procedure. Please follow the above steps as directed.

ADDITION OF ANTI-CONJUGATE IgG PEROXIDASE CONJUGATE, SUBSTRATE AND STOP SOLUTION

h) Using an 8 or 12 channel pipette (or transplating device) dispense 100 µL diluted conjugate (prepared as described above) into each assay well. Discard pipette tips.
i) Incubate for 30 minutes at room temperature.
j) WASH as in steps f and g above.
k) Using an 8 or 12 channel pipette (or transplating device) dispense 100 µL diluted Substrate Solution (prepared as described above) into each test well. Discard pipette tips.
l) Incubate 15 minutes at room temperature.
m) Using an 8 or 12 channel pipette (or transplating device) dispense 100 µL diluted Stop Solution (prepared as described above) into each test well.
n) Allow bubbles to dissipate before reading plate.

MANUAL PROCESSING OF DATA

a) Read the plate using an ELISA plate reader set at 405-410 nm. Be sure to blank the reader as directed.

b) Calculate the average Positive Control Serum absorbance (Optical Density [O.D.]) using the absorbance values of wells A1, A3, and H11. Calculate the Normal Control Serum absorbance using values obtained from wells A2, H10, and H12. Record both averages.

c) Subtract the average normal control absorbance from the average positive absorbance. The difference is the Corrected Positive Control.

d) Calculate a sample to positive (Sp) ratio by subtracting the average normal control absorbance from each sample absorbance. The difference is divided by the corrected positive control. Use the following equation format:

$$SP = \frac{\text{(SAMPLE ABSORBANCE)} - \text{(AVERAGE NORMAL CONTROL ABSORBANCE)}}{\text{CORRECTED POSITIVE CONTROL ABSORBANCE}}$$

e) An ELISA titer for MG/MS can be calculated by the following suggested equation:
LOG_{10} TITER = (1.464 x LOG_{10} Sp) + 3.197
TITER = ANTILOG of LOG_{10} TITER
Example:
1. Example Positive Control Absorbance:
   0.585, 0.610, 0.590
   Average = (0.585 + 0.610 + 0.590) / 3 = 0.595
2. Example Normal Controls:
   0.078, 0.067, 0.057
   Average = (0.078 + 0.067 + 0.057) / 3 = 0.067
   (0.595) – (0.067) = 0.528
4. Example Sp value calculation:
   Absorbance of sample = 0.560
   (0.560) – (0.067) / 0.528 = 0.934
5. Example of Calculation of titer using the Sp from above:
   Log_{10} Titer = 1.464 x (Log_{10} 0.934) + 3.197
   Titer = ANTILOG 3.15
   Titer = 1413

RESULTS

Assay Control Values

Valid ELISA results are obtained when the average optical density (O.D.) value of the Normal Control Serum is less than 0.200 for MG/MS and the corrected Positive Control value is between 0.250 and 1.20. If either of these values are out of range, the test results should be considered invalid and the samples should be retested.

Interpretation of Results

The MG/MS ELISA Sp ratio values and/or ELISA titer values obtained for sera should be interpreted using the following value ranges:

Sample to Positive (Sp) Value	MG/MS ELISA Titer Range	MG/MS Presumed Antibody Status
Less than 0.600	0	Negative[a]
Greater or equal to 0.6	744 or greater	Positive[b]

a. Negative. Serum samples with an MG/MS Sp ratio value of less than 0.600 receive a "0" titer value and are presumed negative for antibody. However, a variety of factors, such as possible strain variations that may exhibit atypical biological and/or antigenic properties[1,2], prevalence of a strain within a flock and timing and randomness of serum sample collection procedures could result in an infected flock yielding negative ELISA results. It is therefore recommended that each flock only be considered to be negative after (a) each flock has been adequately sampled and repeatedly tested several times and has yielded negative ELISA results each time **and** (b) each flock has been adequately sampled and repeatedly tested by standard conventional serological tests (SPA and HI) and MG or MS culture techniques[2,3] and has yielded negative serological and culture results each time.

b. Positive. Additional conventional serologic testing (SPA and HI) and culturing of samples collected from presumed MG/MS ELISA antibody positive flocks, using standard techniques[3,4], are needed to obtain a confirmed positive diagnosis of MG or MS infection within a flock.

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