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Mycoplasma Gallisepticum (MG) Antibody ELISA Test Kit

INSTRUCTION MANUAL

FOR PRODUCT No: LT62003AYSL

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Intended use:

This kit is used to detect Mycoplasma Gallisepticum (MG) virus antibody in chicken serum, to assess antibody condition by Mycoplasma Gallisepticum (MG) vaccine in chicken farm and assist diagnosis of serological infected chicken.

MANUAL VERSION 1.01

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PRINCIPLE

The Mycoplasma Gallisepticum (MG) antibody ELISA kit is based on an indirect enzymatic immunoassay (Indirect ELISA). The antigen is coated on plates. When a sample serum contains specific antibodies against virus, they will bind to the antigen on plates. Wash the unbound antibodies and other components. Then add a specific enzyme conjugate. After incubation and washing, add the TMB substrate. A colorimetric reaction will appear, measured by a spectrophotometer (450 nm).

MATERIALS SUPPLIED WITH THIS KIT

1	MG antigen coated microplate	96T ×1	7	Stopping solution	6 ml
2	Enzyme conjugate	11ml	8	Negative control serum	800µl
3	10X concentrated washing buffer	50ml	9	Positive control serum	800µl
4	Substrate solution A	6 ml	10	Serum dilution plate	1 plate
5	Substrate solution B	6 ml	11	Adhesive film	2 pieces
6	Sample diluent	50ml ×1	12	Instruction	1 piece

MATERIALS REQUIRED BUT NOT PROVIDED

1) Micropipettors and disposable tips: 0.5µl~10µl, 10µl~100µl, 100µl~1000µl.

2) 37 ℃ Incubator.

3) Measuring cylinder: 500 ml.

4) 96 wells microplate reader.

5) Distilled/De-ionized water.

6) Microplate Washer.

SAMPLE PREPARATION

Take animal whole blood, make serum according to regular methods, the serum should be clear, have no hemolysis.

PREPARATION OF WASHING BUFFER

Return washing buffer to room temperature before use, if there are salty crystals, shake to make the crystals dissolve, then use distilled water or deionied water to diluent it at 10 times. The diluent washing buffer can store for 1 week at 4 ℃.

SAMPLE DILUTION

At serum diluent plate, dilute serum at 1:99 with sample dilution (for example: 495μl sample diluent + 5μl serum**)**

Notice: Negative control serum and Positive control serum do not need dilute. Exchange tip after taking sample every time, record the situation of the sample on plate accurately. Shake the sample evenly before adding it.

NOTES

1. All reagents should be adjusted to the room temperature (20-25 ℃) for 30 min to get best results, store at 2-8 ℃ after using.
2. Do not exchange the reagents from the kits of different lot numbers to use. Avoid reagent pollution when using.
3. Substrate and stop solution may have excitant to skin and eyes, pay attention when using.
4. Do not expose TMB (Substrate B) to light and avoid it contact with antioxidants.
5. The wells should avoid damp or touching water after unsealing (Put the un-using

microplate back to bag with dehydrator in 2~8 ℃ soon).

6. Deal all waste reasonable before dumping to avoid pollution.

7. Strictly adhere to instruction to get best result. All procedure including pipetting, timing and washing etc. must be accurate.

8. Serum diluent plate is disposable, do not use for second time; the MAX volume of it is 300μl/well.

PROCEDURE

1. Take pre-coated microplate (Can unseal for several times use as per sample quantity), add 100μl diluted serum to a well, meanwhile set 1 wells for Negative control serum, Positive control serum and blank control wells separately. Add 100 μl Negative/Positive control serum to its wells, only add 100μL sample diluent buffer in the blank control wells. Shake softly, **incubate at 37 ℃ for 30 min**.

2. Pour the liquid out of the wells, add 250 μl diluted washing solution to each well, wait for 1 min, pour out. Repeat 3 times, then tap to dry on absorbent paper.

3. Add 100 μl enzyme conjugate into each well and i**ncubate at 37 ℃ for 30 min**.

4. Repeat step 2 (washing).

5. Add 50 μl substrate A, then substrate B (50 μl) to each well, mix properly, **react for 10 min at dark at 37 ℃ in dark.**

6. Add 50μl stop solution into each well, and measure the OD value of each well at dual-wave length 450nm/630nm.

RESULTS

Set zero at the blank control well, and test the OD450nm (630 nm as reference) value on the microplate-reader. The conditions for the test to be tenable are that the positive control wells' average OD450nm value is greater than or equal to 0.6, and the negative control wells' average OD450nm value is less than 0.15. If the test is invalid, the operation procedure is skeptical, run the test again and observe all the reagents carefully.

If the sample's A450 value is greater than 0.25+ absorbance of negative control, it is judged to be positive; and if less than 0.25+ absorbance of negative control, negative. If absorbance of negative control is less than 0.05, calculate as 0.05

Specifications: 96 wells/kit.

Expiry date: 12 months.

Storage: Storing at 2-8℃, in the dark.