

**MYCOPLASMA GALLISEPTICUM
(MG)ANTIBODY ELISA KIT
MANUAL**

Mycoplasma Gallisepticum (MG) antibody Elisa Test Kit

Catalogue Number. IP100193

Product Usage

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.

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).

Specifications: 96 wells × 2.

Components

	Code item	Spec.
1	MG antigen coated microtiter strips	1/2 pieces
2	Enzyme Conjugate	11/21 mL
3	10× washing solution	50/100 mL
4	Substrate A solution	6/11 mL
5	Substrate B solution	6/11 mL
6	Sample dilution	50/100 mL
7	Stop solution	6/11 mL
8	Negative control	0.8/1.6 mL
9	Positive control	0.8/1.6 mL
10	Serum dilution plate	1/2 pieces
11	Adhesive film	2/4 pieces
	Instruction	1 piece

Material required not provided

1. Micropipettes: 0.5µL~10µL、10µL~100µL、100µL~1000µL
2. Disposable pipette tips
3. Measuring cylinder: 500 ml
4. 96 wells microplate reader

5. Distilled water or deionized water
6. Bottle or microplate washing machine

Sample preparation

Take animal whole blood, get serum by using regular method, the serum should be bright and have no hemolysis.

Washing buffer preparation

Return 10X Concentrated washing buffer to room temperature before use, if there are salt crystals, shake to make it dissolved, then dilute it 10 times with distilled water or deionized water. The diluted washing buffer can be stored at 4°C for about 1 week.

Sample dilution

At serum dilution plate, dilute serum at 1:99 with sample diluent.

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

Operation procedures

1. Adding sample: Take out the required coated plates according to sample quantity (Can be detached) and record the sample position on a worksheet. For single-wave length test, set one blank control well, add nothing; for double-wave length test, do not set blank control well. Set 2 wells for negative control serum and 2 wells for positive control serum, add undiluted negative and positive control serum to its well accordingly, 100 μ L/well. Others are wells for samples, add 100 μ L/well of Sample diluent solution, then add 1 μ L serum sample separately (there will be color change after adding sample).
2. Mix gently for 30s, incubate at 37°C for 30 min.
3. Remove adhesive foil. Pour the liquid out of the wells, add Washing solution(dilute the Wash Concentrate at 20 times with deionized water) into each well fully, stand for 1 min. Repeat 5 times, at last time pat to dry on absorbent paper.
4. Add 100 μ L enzyme conjugate into each well (except the blank well, also do not add any liquid to blank well).
5. Cover plate with new adhesive foil. Incubate at 37 °C for 30 min.
6. Repeat step 3(washing).
7. Add 50 μ L substrate A and substrate 50 μ L B into each well, mix properly, incubate for 15 min at 37 °C in the dark with new adhesive foil.
8. Add 50 μ L stop solution into each well, mix gently and determine the result within 5-30 min.
9. For single-wavelength test, measure the A value with a photometer at 450 nm, set zero for the blank well, and read A value of each well; For double-wavelength test, measure the A value with a photometer at 450 nm (Reference-wavelength: 630 nm), read A value of each well.

Results

Set zero for the blank well, and test the A_{450nm} (630 nm as a reference) value on the microplate-reader. The conditions for the test to be tenable are that the positive control wells' average A_{450nm} value is greater than or equal to 0.6, and the negative control wells' average A_{450nm} value is less

than 0.15. If the test is invalid, the operation is in doubt, retest and observe all the reagents carefully.

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

Notes

- 1) All reagents should be adjusted to the room temperature and shake evenly before using, store at 2-8 °C 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 °C 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 dilution plate is disposable, do not use for second time; the MAX volume of it is 300µL/well.

storage: store at 2-8°C, dark, sealed, dry place, no frozen..

Expiry date: 12 months; date of production is on box.