

**MYCOPLASMA HYOPNEUMONIAE  
(MHP) ANTIBODY ELISA KIT  
MANUAL**

---

## Mycoplasma Hypopneumoniae (MHP) Antibody ELISA Kit

---

**Catalogue Number. IP100180**

### ***Product Usage***

The Mycoplasma hypopneumoniae(MHP) Antibody ELISA test kit is used for the detection of the MHP antibody in swine serum; assessment the immunity conditions against MHP virus, serological diagnosis of pig infection in the pig farms and investigation of the epidemiology of the MHP virus.

### ***Principle***

This kit use indirect ELISA method, MHP antigen is pre-coated on enzyme micro-well strips. When testing, add diluted serum sample, after incubation, if there is MHP specific antibody, it will combine with the pre-coated antigen, discard the uncombined antibody and other components with washing; then add enzyme labeled anti-MHP antibody, combine with the antigen-antibody complex on the plate; discard the uncombined enzyme conjugate with washing; Add TMB substrate in micro-wells, blue product formed enzymatically, after adding stop solution to stop the reaction, use ELISA reader at 450nm wavelength to measure the absorbance A value in reaction wells.

***Specifications:*** 96 wells × 2.

### ***Components***

	<b>Code item</b>	<b>Spec.</b>
1	MHP-Ag Coated plates 96 wells	1/2 plates
2	Enzyme Conjugate	11/22 ml
3	10X Concentrated washing buffer	100 ml
4	Substrate	11/22 ml
5	Sample diluent	100 ml
6	Stop solution	15 ml
7	Negative control	2 ml
8	Positive control	1.6 ml
9	Serum dilution plate	1/2 pieces
10	Adhesive Foil	2/4 pieces
11	Instruction sheet	1 piece

### ***Material required not provided***

1. Micropipettes: 0.5 $\mu$ L~10 $\mu$ L、 10 $\mu$ L~100 $\mu$ L、 100 $\mu$ L~1000 $\mu$ L
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 bright and no hemolysis

### ***Washing buffer preparation***

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

### ***Sample dilution***

At serum dilution plate, dilute serum at 1:100 with sample diluent.

Notice: Negative control and Positive control 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

### ***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  $\mu$ L/well. Others are wells for samples, add 100 $\mu$ L/well of Sample diluent solution, then add 1  $\mu$ 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(dillute 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  $\mu$ L enzyme comjugate 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 $\mu$ L substrate A and substrate 50 $\mu$ L B into each well, mix properly, incubate for 15 min at 37 °C in the dark with new adhesive foil.
8. Add 50 $\mu$ 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**

Read the OD value with ELISA reader at 450nm(630nm as reference)

For the assay to be valid:

OD Value of Negative control (N)< 0.2, meanwhile average OD value of Positive control (P)>0.4.

Calculation method:

Sample OD value / Positive control OD Average value= S/P value

The result is judged by S/P value:

If S/P≥0.5, it is positive; If S/P< 0.5, it is negative.

## **Precautions**

*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) to light and avoid it contact with antioxidants.*

*5) The wells should avoid damp or touching water after unsealing (Put the un-using microtiter strips 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.