



Technical Manual

Human Mycoplasma Pneumoniae (MP) IgG ELISA Kit

- Catalogue Code: HDES0013
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Indirect-ELISA as the principle to detect the Mycoplasma Pneumoniae (MP) IgG in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with purified MP antigen. Samples are added to the ELISA Microtiter plate wells and the MP antibody in which will combine with the pre-coated antigen to form antigen-antibody compound. Free components are washed away. The HRP conjugated Mouse-anti-human IgG antibody is added to each well and react with the compound to form “antigen-antibody-HRP antibody” compound. The TMB substrate is added to initiate the color developing reaction. The presence of MP-IgG can be determined according to the OD value after colorimetric assay with the Micro-plate Reader.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Positive Control	1 mL
Negative Control	1 mL
HRP Conjugate	12 mL
Sample Diluent	12 mL
20×Concentrated Wash Buffer	50 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Plate Sealer	3 pieces
Sealed Bag	1
Manual	1 copy

3. Experimental instrument

- Micro-plate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)
- High-precision transferpettor, EP tubes and disposable pipette tips
- 37° C Incubator or water bath
- Deionized water
- Absorbent paper
- Loading slot for Wash Buffer

4. Sample preparation

1. Fresh collected serum specimens should be fully centrifugal, then take clear liquid for test. The suspended fibrous protein may cause a false positive result if not fully precipitated.
2. Samples can be stored at 2~8° C for one week. If samples not tested in a week, store them below -20° C and avoid freeze-thaw cycles.

5. Assay procedure

Bring all reagents to room temperature for 30 min. Dilute the 20×Concentrated Wash Buffer for 20 times with distilled water.

1. **Add sample:**
 - a) Take out Micro-plate and mark it, reserve 1 well for blank control (empty), 3 wells for negative control, 2 wells for positive control (100 µL control serum for each well). (Blank well is not necessary for dual-wavelength detection)
 - b) Dilute the tested Serum with Sample Diluent at 1:10 into sample well (add 100 µL of Sample Diluent and add 10 µL of serum sample), mix fully.
 - c) Gently tap the plate to ensure thorough mixing.
2. **Incubate:** Cover the ELISA plate with sealer. Incubate for 30 min at 37° C.
3. **Wash:** After incubation, remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 5 times with wash buffer and immerse for 30-60 sec each time.
4. **HRP conjugate:** Add 100 µL of HRP Conjugate working solution to each well except the blank control well.
5. **Incubate:** Cover the ELISA plate with sealer. Incubate for 30 min at 37° C.
6. **Wash:** After incubation, remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 5 times with wash buffer and immerse for 30-60 sec each time.
7. **Add substrate:** Add 50 µL of Substrate Reagent A and 50 µL of Substrate Reagent B to each well. Gently tap the plate to ensure thorough mixing. Cover with a new plate sealer. Incubate for 15 min at 37° C in dark.
8. **Stop reaction:** Add 50 µL of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
9. **OD Measurement:** Set the Micro-plate Reader wavelength at 450 nm (it is recommended to set the dual wavelength at 450 nm/630 nm) to detect A value of each well. Blank well is not needed when using dual wavelength 450 nm/630 nm for detection. **Note: Read the results within 10 min.**

6. Reference value

1. Result analysis

- (1) Use each test result independently. Determine the result according to the Cut Off value.
- (2) Calculate the Cut Off: $\text{Cut Off(C.O)} = 0.10 + \text{negative control (NC) average A value}$ (when NC average $A_{450} < 0.05$, calculate at 0.05; while NC average $A_{450} \geq 0.05$, calculate at the actual value).

2. Quality control

- (1) Blank well (just chromogenic agent and Stop Solution) absorbance ≤ 0.08 .
- (2) Positive control (PC) $A_{450} > 0.80$.
- (3) Negative control (NC) $A_{450} < 0.1$.

The experimental result is valid if quality control is valid.

3. Determination of results

- (1) Positive result: Sample absorbance \geq Cut Off.
- (2) Negative result: Sample absorbance $<$ Cut Off.

7. Interpretation of test results

1. Negative result indicates no MP-IgG antibody detected in samples, while positive result is just the opposite.
2. The positive result of MP-IgG antibody is an important index of MP infection.

8. Limitations of test method

1. All high sensitivity immune experiment system exists potential non-specificity. Therefore, unacceptable positive results may be caused by biological false positive of ELISA method.
2. Any positive result should be combined with clinical information to determine the result.

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