Instructions for Use

For the qualitative determination of IgM class antibodies against *Mycoplasma pneumoniae* in Human serum or plasma (citrate)

This product is for research use only and is not intended for diagnostic use.
1. Introduction

ab108756 *Mycoplasma pneumoniae* IgM Human ELISA Kit is intended for the qualitative determination of IgM class antibodies against *Mycoplasma pneumoniae* in human serum or plasma (citrate).

The mycoplasms belong to the class Mollicutes comprising three distinct families and four genera, one of which is Mycoplasma with over 60 species. Mycoplasma are the smallest free living organisms known (300 to 500 nm in diameter) and unlike regular bacteria they lack a cell wall. Mycoplasms are extracellular parasites, especially on mucous membranes, which can cause infections in humans, animals, plants, and cell cultures. *Mycoplasma pneumoniae* is primarily a respiratory pathogen (obligate) in humans involving the nasopharynx, throat, trachea, bronchi, bronchioles, and alveoli. Other Mycoplasma, *M. buccale, M. faecium, M. orale* and *M. salivarium* are commensals in the oral cavity. *Mycoplasma hominis* and *Ureaplasma urealyticum* inhabit primarily the genital tract and may act as opportunistic invaders. *M. pneumoniae* is by far the most important pathogen of this group. Infection with *M. pneumoniae* occurs worldwide. It has been estimated to cause 15-20% of all pneumonia; the rate is highest in children and young adults. 74% of infections with *M. pneumoniae* are asymptomatic.
2. Assay Summary

*ab108756 is for the qualitative immunoenzymatic determination of IgM-class antibodies against Mycoplasma pneumoniae and is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.*

Microtiter strip wells are precoated with *Mycoplasma pneumoniae* antigens to bind corresponding antibodies of the specimen. Incubate 1 hour.

\[\text{\downarrow}\]

After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labeled anti-human IgM conjugate is added. Incubate for 30 minutes. This conjugate binds to the captured *Mycoplasma pneumoniae*-specific antibodies.

\[\text{\downarrow}\]

The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. Incubate 15 minutes. The intensity of this product is proportional to the amount of *Mycoplasma pneumoniae*-specific IgM antibodies in the specimen.

\[\text{\downarrow}\]

Sulphuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorption at 450 nm is read using an ELISA microwell plate reader.
3. Kit Contents

- **Mycoplasma pneumoniae Coated Wells (IgM)**: 12 break apart 8-well snap-off strips coated with Mycoplasma pneumoniae antigen; in resealable aluminium foil.
- **IgM Sample Diluent***: 1 bottle containing 100 ml of buffer for sample dilution; pH 7.2 ± 0.2; colored green; ready to use; white cap.
- **Stop Solution**: 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Solution (20x conc.)***: 1 bottle containing 50 ml of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.
- **Mycoplasma pneumoniae anti-IgM Conjugate**: 1 bottle containing 20 ml of peroxidase labeled rabbit antibody to human IgM; colored red, ready to use; black cap.
- **TMB Substrate Solution**: 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; yellow cap.
- **Mycoplasma pneumoniae IgM Positive Control***: 1 bottle containing 2 ml; colored yellow; ready to use; red cap.
- **Mycoplasma pneumoniae IgM Cut-off Control***: 1 bottle containing 3 ml; colored yellow; ready to use; green cap.
- **Mycoplasma pneumoniae IgM Negative Control***: 1 bottle containing 2 ml; colored yellow; ready to use; blue cap.
- **Strip holder**: 1
• **Cover foil**: 1
  
  * contains 0.1 % Bronidox L after dilution
  ** contains 0.2 % Bronidox L
  *** contains 0.1 % Kathon

4. **Storage and Handling**

The reagents are stable up to the expiry date stated on the label when stored at 2-8 °C.

5. **Additional Materials Required**

• ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
• Incubator 37°C
• Manual or automatic equipment for rinsing wells
• Pipettes to deliver volumes between 10 and 1000 µl
• Vortex tube mixer
• Deionised or (freshly) distilled water
• Disposable tubes
• Timer
6. Preparation of Reagents

*It is very important to bring all reagents, samples and standards to room temperature (20-25 °C) before starting the test run.*

1. **Coated snap-off Strips:** The ready to use break apart snap-off strips are coated with Mycoplasma pneumoniae antigen. Store at 2-8°C. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2-8 °C; stability until expiry date.*

2. **Mycoplasma pneumoniae anti-IgM Conjugate:** The bottle contains 20 ml of a solution with anti-human-IgM horseradish peroxidase, buffer, stabilizers, preservatives and an inert red dye. The solution is ready to use. Store at 2-8°C. *After first opening stability until expiry date when stored at 2-8 °C.*

3. **Controls:** The bottles labeled with Positive, Cut-off and Negative Control contain a ready to use control solution. It contains 0.1% Kathon and has to be stored at 2-8°C. *After first opening stability until expiry date when stored at 2-8 °C.*

4. **IgM Sample Diluent:** The bottle contains 100 ml phosphate buffer, anti-human-IgG, stabilizers, preservatives and an inert green dye. It is used for the dilution of the specimen. The solution contains anti-human IgG class antibodies to eliminate competitive inhibition from specific IgG class antibody to remove rheumatoid factor. This ready to use solution has to be
stored at 2-8°C. *After first opening stability until expiry date when stored at 2-8 °C.*

5. **Washing Solution (20x conc.):** The bottle contains 50 ml of a concentrated buffer, detergents and preservatives. Dilute Washing Solution 1+19; e.g. 10 ml Washing Solution + 190 ml fresh and germ free redistilled water. The diluted buffer will keep for 5 days if stored at room temperature. *Crystals in the solution disappear by warming up to 37°C in a water bath. After first opening the concentrate is stable until the expiry date.*

6. **TMB Substrate Solution:** The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2-8°C away from the light. *The solution should be colorless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away. After first opening stability until expiry date when stored at 2-8°C.*

7. **Stop Solution:** The bottle contains 15 ml 0.2 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2-8°C. *After first opening stability until expiry date.*
7. Preparation and Collection of Specimen

Use human serum or plasma (citrate) samples with this assay. If the assay is performed within 5 days after sample collection, the specimen should be kept at 2-8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.

Heat inactivation of samples is not recommended.

Sample Dilution:

Before assaying, all samples should be diluted 1+100 with IgM Sample Diluent. Dispense 10µl sample and 1ml IgM Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. Assay Method

Test Preparation

Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. If performing the test on ELISA automatic systems we recommend to increase the washing steps from three to five and the
volume of washing solution from 300µl to 350µl to avoid washing effects. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established. Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:

- 1 well (e.g. A1) for the blank
- 1 well (e.g. B1) for the negative control
- 2 wells (e.g. C1+D1) for the cut-off control and
- 1 well (e.g. E1) for the positive control

*It is recommended to determine controls and samples in duplicate, if necessary.*

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each standard and each sample.

Adjust the incubator to 37° ± 1°C.

**Assay Procedure:**

1. Dispense 100 µl controls and diluted samples into their respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37±1°C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with
300µl of Washing Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

*Note:* Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.

5. Dispense 100µl Mycoplasma pneumoniae anti-IgM Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.

6. **Incubate for 30 min at room temperature.** *Do not expose to direct sunlight.*

7. Repeat step 4.

8. Dispense 100µl TMB Substrate Solution into all wells.

9. **Incubate for exactly 15 min at room temperature in the dark.**

10. Dispense 100µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. *Any blue colour developed during the incubation turns into yellow.*

    *Note:* Highly positive samples can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. Predilution of the sample with physiological sodium chloride solution, for example 1+1, is recommended. Then dilute the sample 1+100 with dilution buffer and multiply the results in NTU by 2.

11. Measure the absorbance of the specimen at 450/620 nm within 30 min after addition of the Stop Solution.
**Measurement:**

Adjust the ELISA Microwell Plate Reader **to zero** using the **substrate blank in well A1.**

*If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!*  

**Measure the absorbance** of all wells at **450 nm** and record the absorbance values for each standard and sample.

*Dual wavelength reading using 620 nm as reference wavelength is recommended.*

Where applicable calculate the **mean absorbance values** of all duplicates.
9. Data Analysis

A. Run Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

Substrate blank in A1: Absorbance value < 0.100.
Negative control in B1: Absorbance value < 0.200 and < cut-off.
Cut-off control in C1 and D1: Absorbance value 0.150 – 1.300.
Positive control in E1: Absorbance value > cut-off.

If these criteria are not met, the test is not valid and must be repeated.

B. Calculation of Results

The cut-off is the mean absorbance value of the Cut-off control determinations.

Example: Absorbance value Cut-off control 0.49 + absorbance value Cut-off control 0.47 =0.96 / 2 = 0.48

Cut-off = 0.48

C. Interpretation of Results
Samples are considered **POSITIVE** if the absorbance value is higher than 10% over the cut-off.

Samples with an absorbance value of 10% above or below the cut-off should not be considered as clearly positive or negative → grey zone

It is recommended to repeat the test again 2 - 4 weeks later with a fresh sample. If results in the second test are again in the grey zone the sample has to be considered **NEGATIVE**.

Samples are considered **NEGATIVE** if the absorbance value is lower than 10% below the cut-off.

**D. Results in Abcam Units**

\[
\text{Sample (mean) absorbance value} \times 10 = [\text{Abcam Units} = \text{NTU}]
\]

\[
\text{Cut off}
\]

*Example:* \[
1.786 \times 10 = 37 \text{ NTU} [\text{Abcam Units} = \text{NTU}]
\]

\[
0.48
\]

<table>
<thead>
<tr>
<th>Cut-off:</th>
<th>10</th>
<th>NTU</th>
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</thead>
<tbody>
<tr>
<td>Grey zone:</td>
<td>9-11</td>
<td>NTU</td>
</tr>
<tr>
<td>Negative:</td>
<td>&lt;9</td>
<td>NTU</td>
</tr>
<tr>
<td>Positive:</td>
<td>&gt;11</td>
<td>NTU</td>
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</tbody>
</table>
E. Precision

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<thead>
<tr>
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</thead>
<tbody>
<tr>
<td><strong>Inter-assay</strong></td>
<td><strong>n</strong></td>
<td><strong>Mean (NTU)</strong></td>
<td><strong>Cv (%)</strong></td>
</tr>
<tr>
<td>Pos. Serum</td>
<td>12</td>
<td>21.6</td>
<td>4.8</td>
</tr>
<tr>
<td>Pos. Serum</td>
<td>12</td>
<td>13.3</td>
<td>7.8</td>
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<thead>
<tr>
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<tbody>
<tr>
<td><strong>Intra-assay</strong></td>
<td><strong>n</strong></td>
<td><strong>Mean (E)</strong></td>
<td><strong>Cv (%)</strong></td>
</tr>
<tr>
<td>Pos. Serum</td>
<td>31</td>
<td>0.99</td>
<td>2.9</td>
</tr>
<tr>
<td>Pos. Serum</td>
<td>22</td>
<td>0.76</td>
<td>9.2</td>
</tr>
</tbody>
</table>

F. Specificity

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is > 95 %.

G. Sensitivity

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 94.4 %.

H. Interferences

Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

**Note:** The results refer to the groups of samples investigated; these are not guaranteed specifications.
10. Limitations

- Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.
### 11. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Confirm dilutions made correctly</td>
</tr>
<tr>
<td></td>
<td>Standard improperly reconstituted (if applicable)</td>
<td>Briefly spin vial before opening; thoroughly resuspend powder (if applicable)</td>
</tr>
<tr>
<td></td>
<td>Standard degraded</td>
<td>Store sample as recommended</td>
</tr>
<tr>
<td></td>
<td>Curve doesn't fit scale</td>
<td>Try plotting using different scale</td>
</tr>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Target present below detection limits of assay</td>
<td>Decrease dilution factor; concentrate samples</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when concentration of target is too high</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>High background</td>
<td>Wells are insufficiently washed</td>
<td>Wash wells as per protocol recommendations</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
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<tr>
<td></td>
<td>Waiting too long to read plate after adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
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<td>------------------------</td>
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<td>-----------------------------------------------</td>
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<tr>
<td></td>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
</tr>
<tr>
<td></td>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
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<tr>
<td></td>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
</tr>
<tr>
<td></td>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. Serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
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For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “contact us” on www.abcam.com for the phone number for your region).