

Product information

Information about other products is available at: www.demeditec.com



User's Manual

Mycoplasma pneumo- niae IgG ELISA



DEMYCG0350



96 wells



Demeditec Diagnostics GmbH
Lise-Meitner-Strasse 2
24145 Kiel – Germany
www.demeditec.com

CONTENTS

| | |
|--|----|
| 1. INTRODUCTION | 3 |
| 2. INTENDED USE | 3 |
| 3. PRINCIPLE OF THE ASSAY | 3 |
| 4. MATERIALS | 4 |
| 5. STABILITY AND STORAGE | 4 |
| 6. REAGENT PREPARATION | 4 |
| 7. SAMPLE COLLECTION AND PREPARATION | 5 |
| 8. ASSAY PROCEDURE | 5 |
| 9. RESULTS | 6 |
| 10. SPECIFIC PERFORMANCE CHARACTERISTICS | 7 |
| 11. LIMITATIONS OF THE PROCEDURE | 7 |
| 12. PRECAUTIONS AND WARNINGS | 8 |
| BIBLIOGRAPHY | 9 |
| ABBREVIATIONS | 9 |
| SUMMARY OF TEST PROCEDURE | 10 |
| SYMBOLS USED WITH DEMEDITEC ASSAYS | 12 |

1. INTRODUCTION

The mycoplasmas belong to the class Mollicutes comprising three distinct families and four genera, one of which is Mycoplasma with over 60 species. Mycoplasmas are the smallest free living organisms known (300 to 500 nm in diameter) and unlike regular bacteria they lack a cell wall. Mycoplasmas are extracellular parasites, especially on mucous membranes, which can cause infections in human, animals, plants, and cell cultures. Mycoplasma pneumoniae is primarily a respiratory pathogen (obligat) in human involving the nasopharynx, throat, trachea, bronchi, bronchioles, and alveoli. Other Mycoplasmas, M. buccale, M. faucium, 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, its epidemiology has been studied primarily in the USA, Europe, and Japan. Infections are endemic in larger urban areas, and epidemic increases are observed at varying intervals. M. pneumoniae has been estimated to cause 15-20% of all pneumoniae; the rate is highest in children and young adults. 74% of infections with M. pneumoniae are asymptomatic, reinfection may occur. Naturally acquired immunity to infection with M. pneumoniae appears to be of limited duration (2-3 years).

| Species | Disease | Symptoms (e.g.) | Transmission route |
|---------------|---|--|---------------------------------|
| M. pneumoniae | Respiratory diseases by Mycoplasma pneumoniae | Fever, headache, and a persistent cough. Respiratory tract disease: from asymptomatic infection to colds, pharyngitis, bronchitis, croup, tracheobronchitis, pneumonitis and primary atypical pneumonia | Transmitted by aerosol droplets |

Infection or presence of pathogen may be identified by:

- Microscopy
- Serology: e.g. by ELISA

2. INTENDED USE

The Mycoplasma pneumoniae IgG ELISA is intended for the qualitative determination of IgG class antibodies against Mycoplasma pneumoniae in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

1. **SORB** **MT** **Microtiterplate**: 12 break-apart 8-well snap-off strips coated with Mycoplasma pneumoniae antigens; in resealable aluminium foil.
2. **SAM** **DIL** **IgG Sample Dilution Buffer**: 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2 ; coloured yellow; ready to use; white cap; $\leq 0.0015\%$ (v/v) CMIT/ MIT (3:1).
3. **STOP** **SOLN** **Stop Solution**: 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
4. **WASH** **SOLN** **20x** **Washing Buffer (20x conc.)**: 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2 , for washing the wells; white cap.
5. **ENZ** **CONJ** **Conjugate**: 1 bottle containing 20 mL of peroxidase labelled antibody to human in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
6. **SUB** **TMB** **TMB Substrate Solution**: 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), $< 0.1\%$; ready to use; yellow cap.
7. **CAL** **C** **Positive Control**: 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; $\leq 0.02\%$ (v/v) MIT.
8. **CAL** **B** **Cut-off Control**: 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; $\leq 0.02\%$ (v/v) MIT.
9. **CAL** **A** **Negative Control**: 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; $\leq 0.0015\%$ (v/v) CMIT/ MIT (3:1).

Controls are calibrated in arbitrary units against internal quality control specimens, since no international standard reference is available for this assay.

For hazard and precautionary statements see 12.1

For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 μL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

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

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20...25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Mycoplasma pneumoniae antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20...25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70...-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing. Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

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

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

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the 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 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value 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/control and sample in the-plate layout. Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank:** Absorbance value < **0.100**
- **Negative Control:** Absorbance value < **0.200** and < **Cut-off**
- **Cut-off Control:** Absorbance value **0.150 – 1.300**
- **Positive Control:** Absorbance value > **Cut-off**

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

9.2. Calculation of Results

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

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [U]

$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = [\text{Units} = \text{U}]$

Example: $\frac{1.591 \times 10}{0.43} = 37 \text{ U (Units)}$

9.3. Interpretation of Results

| | | |
|---|----------|--|
| Cut-off | 10 U | - |
| Positive | > 11 U | Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine). |
| Equivocal | 9 – 11 U | Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as negative . |
| Negative | < 9 U | The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely. |
| Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value. | | |

9.3.1. Antibody Isotypes and State of Infection

| Serology | Significance |
|----------|---|
| IgM | Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM |
| IgG | Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection |
| IgA | Produced in mucosal linings throughout the body (⇒ protective barrier) Usually produced early in the course of the infection |

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications. For further information about the specific performance characteristics please contact Demeditec Diagnostics GmbH.

10.1. Precision

| Intraassay | n | Mean (E) | CV (%) |
|-------------------|----------|-----------------|---------------|
| #1 | 24 | 0.434 | 3.80 |
| #2 | 24 | 1.240 | 4.08 |
| #3 | 24 | 1.635 | 6.03 |
| Interassay | n | Mean (U) | CV (%) |
| #1 | 12 | 32.58 | 5.73 |
| #2 | 12 | 31.56 | 13.32 |
| #3 | 12 | 4.36 | 11.10 |

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.

It is 97.8% (95% confidence interval: 92.29% - 99.73%).

10.3. Diagnostic Sensitivity

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

It is 100% (95% confidence interval: 96.87% - 100%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (see 4.1 oder refers to 4.1)

Therefore, the following hazard and precautionary statements apply.



| | |
|-----------|---|
| H317 | May cause an allergic skin reaction. |
| P261 | Avoid breathing spray |
| P280 | Wear protective gloves/ protective clothing. |
| P302+P352 | IF ON SKIN: Wash with plenty of soap and water. |
| P333+P313 | If skin irritation or rash occurs: Get medical advice/ attention. |
| P362+P364 | Take off contaminated and Wash it before reuse. |

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

BIBLIOGRAPHY

Mycoplasmataceae (2009). In Herbert Hof, Rüdiger Dörries, Gernot Geginat: Medizinische Mikrobiologie. [Immunologie, Virologie, Bakteriologie, Mykologie, Parasitologie, klinische Infektiologie, Hygiene] ; 237 Tabellen. 4., vollst. überarb. und erw. Aufl. Stuttgart: Thieme (Duale Reihe), pp. 452–455.

Clyde, Wallace A., JR (1993): Clinical overview of typical Mycoplasma pneumoniae infections. In *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 17 Suppl 1, S32-6.

Foy, Hjordis M. (1993): Infections caused by Mycoplasma pneumoniae and possible carrier state in different populations of patients. In *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 17 Suppl 1, S37-46.

Jacobs, E. (1993): Serological diagnosis of Mycoplasma pneumoniae infections: a critical review of current procedures. In *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 17 Suppl 1, S79-82.

Kayser, Fritz H. (2005): Bacteria as Human Pathogens. In Fritz H. Kayser, Kurt A. Bienz, Johannes Eckert, Rolf M. Zinkernagel: Medical microbiology. Stuttgart, New York: Thieme (Thieme Flexibook), pp. 229–346.

O'Handley, John G.; Gray, Larry D. (1997): The incidence of Mycoplasma pneumoniae pneumonia. In *The Journal of the American Board of Family Practice* 10 (6), pp. 425–429.

Vikerfors, Tomas; Brodin, Glenn; Grandien, Monica; Hirschberg, Lotta; Krook, Aud; Pettersson, Carl-Axel (1988): Detection of specific IgM antibodies for the diagnosis of Mycoplasma pneumoniae infections: a clinical evaluation. In *Scandinavian journal of infectious diseases* 20 (6), pp. 601–610.

Wiegand, R. (1994): Mycoplasma pneumoniae. In Henning Brandis, Hans J. Eggers, W. Köhler, Gerhard Pulverer (Eds.): Lehrbuch der medizinischen Mikrobiologie. 7. Aufl. Stuttgart: Fischer; G. Fischer.

ABBREVIATIONS

| | |
|------|--|
| CMIT | 5-chloro-2-methyl-4-isothiazolin-3-one |
| MIT | 2-methyl-2H-isothiazol-3-one |












SUMMARY OF TEST PROCEDURE**SCHEME OF THE ASSAY**
Mycoplasma pneumoniae IgG ELISA**Test Preparation**

Prepare reagents and samples as described.
Establish the distribution and identification plan for all samples and standards/controls on the plate layout supplied in the kit.
Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

| | Substrate Blank (A1) | Negative Control | Cut-off Control | Positive Control | Sample (diluted 1+100) |
|---|----------------------|------------------|-----------------|------------------|------------------------|
| Negative Control | - | 100 µL | - | - | - |
| Cut-off Control | - | - | 100 µL | - | - |
| Positive Control | - | - | - | 100 µL | - |
| Sample (diluted 1+100) | - | - | - | - | 100 µL |
| Cover wells with foil supplied in the kit Incubate for 1 h at 37 ± 1 °C Wash each well three times with 300 µL of Washing Buffer | | | | | |
| Conjugate | - | 100 µL | 100 µL | 100 µL | 100 µL |
| Incubate for 30 min at room temperature (20...25 °C) Do not expose to direct sunlight Wash each well three times with 300 µL of Washing Buffer | | | | | |
| TMB Substrate Solution | 100 µL | 100 µL | 100 µL | 100 µL | 100 µL |
| Incubate for exactly 15 min at room temperature (20...25 °C) in the dark | | | | | |
| Stop Solution | 100 µL | 100 µL | 100 µL | 100 µL | 100 µL |
| Photometric measurement at 450 nm (reference wavelength: 620 nm) | | | | | |

SYMBOLS USED WITH DEMEDITEC ASSAYS

| Symbol | English | Deutsch | Français | Espanol | Italiano |
|---|------------------------------------|--|--|---|-------------------------------------|
|  | European Conformity | CE-Konformitätskennzeichnung | Conforme aux normes européennes | Conformidad europea | Conformità europea |
|  | Consult instructions for use | Gebrauchsanweisung beachten | Consulter les instructions d'utilisation | Consulte las Instrucciones | Consultare le istruzioni per l'uso |
|  | In vitro diagnostic device | In-vitro-Diagnostikum | Usage Diagnostic in vitro | Diagnóstico in vitro | Per uso Diagnostica in vitro |
|  | For research use only | Nur für Forschungszwecke | Seulement dans le cadre de recherches | Sólo para uso en investigación | Solo a scopo di ricerca |
|  | Catalogue number | Katalog-Nr. | Référence | Número de catálogo | No. di Cat. |
|  | Lot. No. / Batch code | Chargen-Nr. | No. de lot | Número de lote | Lotto no |
|  | Contains sufficient for <n> tests/ | Ausreichend für "n" Ansätze | Contenu suffisant pour "n" tests | Contenido suficiente para <n> ensayos | Contenuto sufficiente per "n" saggi |
|  | Note warnings and precautions | Warnhinweise und Vorsichtsmaßnahmen beachten | Avertissements et mesures de précaution font attention | Tiene en cuenta advertencias y precauciones | Annoti avvisi e le precauzioni |
|  | Storage Temperature | Lagerungstemperatur | Temperature de conservation | Temperatura de conservación | Temperatura di conservazione |
|  | Expiration Date | Mindesthaltbarkeitsdatum | Date limite d'utilisation | Fecha de caducidad | Data di scadenza |
|  | Legal Manufacturer | Hersteller | Fabricant | Fabricante | Fabbricante |
| <i>Distributed by</i> | Distributor | Vertreiber | Distributeur | Distribuidor | Distributore |