Version 22-04/24

User's Manual

Mycoplasma pneumoniae IgG ELISA Kit

REF **DEIA366**

Σ **96T**

RUO

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human antibodies of the immunoglobulin class IgG against Mycoplasma pneumoniae in serum or plasma.

Principles of Testing

The test kit contains microtiter strips each with 8 break-off reagent wells coated with Mycoplasma pneumoniae antigens. In the first reaction step, diluted samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Reagents And Materials Provided

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator 1 200 RU/ml (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL 1
3. Calibrator 2 20 RU/ml (IgG, human), ready for use	red	1 x 2.0 ml	CAL 2
4. Calibrator 3 2 RU/ml (IgG, human), ready for use	light red	1 x 2.0 ml	CAL 3
5. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
6. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
7. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
8. Sample buffer ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
10. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
12. Test instruction	---	1 booklet	
13. Quality control certificate	---	1 protocol	

Materials Required But Not Supplied

1. Microplate reader
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Storage

The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Specimen Collection And Preparation

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Samples are diluted 1:101 sample buffer. For example: dilute 10 µl sample in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: The calibrators and controls are prediluted and ready for use, do not dilute them.

Plate Preparation

The pipetting protocol for microtiter strips 1 to 4 is an example for the semiquantitative analysis of 24 samples (P 1 to P 24).

The pipetting protocol for microtiter strips 7 to 10 is an example for the quantitative analysis of 24 samples (P 1 to P 24).

The calibrators (C 1 to C 3), the positive (pos.) and negative (neg.) controls, and the samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.

The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimizes reagent wastage.

Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	C 2	P 6	P 14	P 22			C 1	P 4	P 12	P 20		
B	pos.	P 7	P 15	P 23			C 2	P 5	P 13	P 21		
C	neg.	P 8	P 16	P 24			C 3	P 6	P 14	P 22		
D	P 1	P 9	P 17				pos.	P 7	P 15	P 23		
E	P 2	P 10	P 18				neg.	P 8	P 16	P 24		
F	P 3	P 11	P 19				P 1	P 9	P 17			
G	P 4	P 12	P 20				P 2	P 10	P 18			
H	P 5	P 13	P 21				P 3	P 11	P 19			

Reagent Preparation

All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

Coated wells: Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag). Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

Calibrators and controls: Ready for use. The reagents must be mixed thoroughly before use.

Enzyme conjugate: Ready for use. The enzyme conjugate must be mixed thoroughly before use.

Sample buffer: Ready for use.

Wash buffer: The wash buffer is a 10× concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water). For example: For 1 microplate strip: 5 ml concentrate plus 45 ml water. The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

Chromogen/substrate solution: Ready for use. Close the bottle immediately after use, as the contents are sensitive to light . The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

Stop solution: Ready for use.

Assay Procedure

Washing:

Manual: Remove the protective foil, empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.

Automatic: Remove the protective foil and wash the reagent wells 3 times with 450 µl of working strength wash buffer.

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values. Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Procedure:

1. Sample incubation: Transfer 100 µl of the calibrators, positive and negative controls or diluted samples into the individual microplate wells according to the pipetting protocol. Incubate for 30 minutes at room temperature (+18°C to +25°C).
2. Washing: Empty the wells. Wash as described above.
3. Conjugate incubation: Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells. Incubate for 30 minutes at room temperature (+18°C to +25°C).
4. Washing: Empty the wells. Wash as described above.
5. Substrate incubation: Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for 15 minutes at room temperature (+18°C to +25°C; protect from direct sunlight).
6. Stopping: Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.
7. Measurement: Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm within 30 minutes of adding the stop solution. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Calculation

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or sample over the extinction value of the calibrator 2. Calculate the ratio according to the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

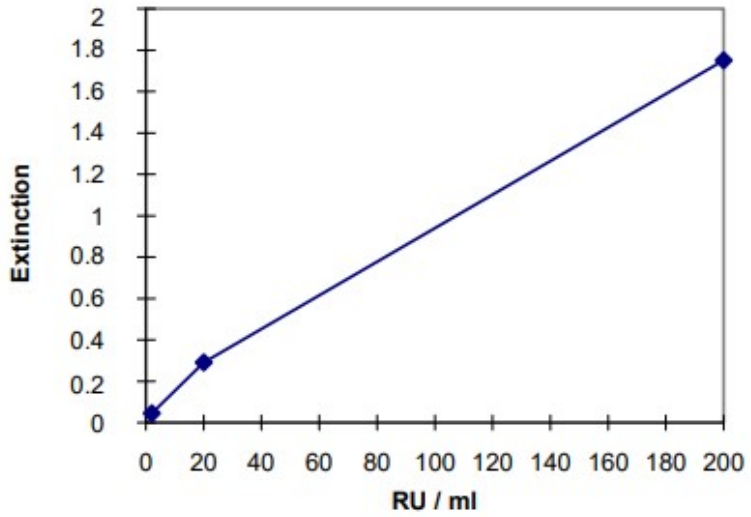
We recommends interpreting results as follows:

Ratio <0.8: negative

Ratio ≥0.8 to <1.1: borderline

Ratio ≥1.1: positive

Quantitative: The standard curve from which the concentration of antibodies in the samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 3 calibration sera against the corresponding units (linear/linear). Use "point-to-point" plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in samples.



If the extinction for a sample lies above the value of calibrator 1 (200 RU/ml), the result should be reported as ">200 RU/ml". It is recommended that the sample be re-tested at a dilution of e.g. 1:400. The result in RU/ml read from the calibration curve for this sample must then be multiplied by a factor of 4.

The upper limit of the normal range of non-infected persons (cut-off value) recommended by CD is 20 relative units (RU/ml). We recommends interpreting results as follows:

<16 RU/ml: negative

≥16 to <22 RU/ml: borderline

≥22 RU/ml: positive

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, We recommends retesting the samples.

Performance Characteristics

Calibration: As no international reference serum exists for antibodies against Mycoplasma pneumoniae, the calibration is performed in relative units (RU). For every group of tests performed, the extinction values of the calibrators and the relative units and/or ratios determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated. The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18°C to +25°C) during the incubation steps, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The antigen is a detergent extract of Mycoplasma pneumoniae, strain MAC ATCC 15531. The extract was further purified to prevent cross reactivity.

Detection Limit

The lower detection limit is defined as mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The detection limit of the Mycoplasma pneumoniae IgG ELISA Kit is 1 RU/ml.

Specificity

The quality of the antigen used ensures a high specificity and sensitiviy of the ELISA. Sera from samples with infections caused by various agents were investigated with the Mycoplasma pneumoniae IgG ELISA Kit. This ELISA showed no cross reactivity.

Antibodies against	n	Anti-Mycoplasma pneumoniae ELISA (IgG) positive
Adenovirus	12	0%
Chlamydia pneumoniae	6	0%
CMV	7	0%
EBV-CA	12	0%
Helicobacter pylori	8	0%
HSV-1	9	0%
Influenza virus A	11	0%
Influenza virus B	12	0%
Measles virus	12	0%
Mumps virus	12	0%
Parainfluenza virus Pool	12	0%
Rubella virus	12	0%
RSV	12	0%
Toxoplasma gondii	3	0%
VZV	11	0%

Linearity

The linearity of the Mycoplasma pneumoniae IgG ELISA Kit was determined by assaying 4 serial dilutions of different samples. The coefficient of determination R^2 for all sera was> 0.95. The Mycoplasma pneumoniae IgG ELISA Kit is linear at least in the tested concentration range (9 RU/ml to 153 RU/ml).

Reproducibility

The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 sera with values at different points on the calibration curve. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed on 6 different runs.

Intra-assay variation, n = 20		
Serum	Mean value (RU/ml)	CV (%)
1	23	4.4
2	38	6.3
3	71	7.1

Inter-assay variation, n = 4 x 6		
Serum	Mean value (RU/ml)	CV (%)
1	22	9.2
2	42	7.0
3	71	8.5

Interferences

Haemolytic, lipaemic and icteric samples showed no influence at the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Precautions

1. Samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents are to be disposed of according to official regulations.
2. The calibrators and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.