

Version 1 Last updated 21 March 2019

ab247197 *Legionella* *Pneumophila* IgG ELISA kit

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For the qualitative determination of IgG class antibodies against *Legionella Pneumophila* in human serum or plasma (citrate, heparin).

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

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1. Overview

Legionella pneumophila IgG ELISA kit (ab247197) is designed for the qualitative determination of IgG class antibodies against *Legionella pneumophila* in human serum or plasma (citrate, heparin).

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microplates 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 microwell plate reader.

Legionellae are aerobic gram-negative facultative intracellular parasites of certain protozoa. They are found in freshwater environments worldwide and can cause respiratory disease (legionellosis) in humans. *L. pneumophila* multiplies itself at temperatures between 25 and 42 °C, with an optimal growth temperature of 35 °C. *Legionella* thrives in warm, stagnant water in the environment and in artificial systems such as cooling towers, evaporative condensers, hot and cold water systems and spa pools that mimic the natural environment in which the organism thrives. These systems also provide the means by which aerosols/droplets are generated and the organism dispersed into the atmosphere.

Legionellosis can be acquired by the inhalation of aerosols containing *Legionella* bacteria or by micro-aspiration of ingested water contaminated with *Legionella*. Person-to-person transmission is not thought to be a risk.

2. Protocol Summary

Prepare all reagents, samples, and controls as instructed



Add 100 μ L control or sample to appropriate wells



Incubate for 1 hour at 37°C



Aspirate and wash each well three times with 300 μ L 1X Washing
Solution



Add 100 μ L of conjugate to each well. Incubate for 30 minutes at
room temperature. Repeat the washing steps.



Add 100 μ L TMB Substrate Solution to each well and incubate for 15
minutes at room temperature in the dark.



Add 100 μ L Stop Solution and read OD at 450/620 nm within 30
minutes after addition of the stop solution.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
20X Washing Solution	50 mL	+4°C
Cover Foil	1 unit	+4°C
Stop Solution	15 mL	+4°C
TMB Substrate Solution	15 mL	+4°C
Legionella pneumophila Coated Microplate (IgG)	1 unit	+4°C
Legionella pneumophila anti-IgG HRP conjugate	20 mL	+4°C
IgG Cut-off Control	3 mL	+4°C
IgG Negative Control	2 mL	+4°C
IgG Positive Control	2 mL	+4°C
IgG Sample Diluent	100 mL	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450/620 nm.
- Deionized water.
- Multi- and single-channel pipettes.
- Plate shaker for all incubation steps.

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 20X Washing Solution:

Prepare 1X Washing Solution by diluting 20X Washing Solution with deionized water. To make 200 mL combine 10 mL 20X with 190 mL deionized water. Mix thoroughly and gently. The diluted buffer is stable for 5 days at room temperature. In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

9.2 *Legionella pneumophila* Coated Microplate (IgG):

The break-apart snap-off strips are coated with *Legionella pneumophila* antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminum foil along with the desiccant supplied and stored at 4 °C.

9.3 TMB Substrate Solution:

The reagent is ready to use and has to be stored at 4 °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.

10. Sample 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 4°C; otherwise they should be aliquoted and stored deep-frozen (-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.

11. Sample Dilution

- Before assaying, all samples should be diluted 1:100 with IgG Sample Diluent. Dispense 10 µL sample and 990 µL IgG Sample Diluent into tubes to obtain a 1:100 dilution and thoroughly mix with a Vortex.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
 - We recommend that you assay all controls and samples in duplicate.
- 12.1** Prepare all reagents, controls and samples as directed in the previous sections.
 - 12.2** Use the plate layout to plan the location for all controls and samples.
 - 12.3** Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
 - 12.4** Add 100 µL of all diluted samples and controls to appropriate wells. Leave well A1 as the Substrate blank. Cover the wells with the cover foil.
 - 12.5** Incubate for 1 hour at 37°C.
 - 12.6** When incubation is completed, remove the foil, aspirate the content of the wells and wash each well 3 x 300 µL with 1X Washing Solution. Avoid overflows from the reaction wells. The interval between washing and aspiration should be less than 5 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.
 - 12.7** Add 100 µL of conjugate to each well, except A1, and incubate for 30 minutes in the dark at room temperature.
 - 12.8** Repeat the washing steps as per 12.6.
 - 12.9** Add 100 µL of TMB Substrate Solution to each well. Incubate for exactly 15 minutes at room temperature in the dark. A blue color occurs due to an enzymatic reaction.
 - 12.10** Add 100 µL of Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a color change from blue to yellow occurs.
 - 12.11** Measure the absorbance at 450/620 nm within 30 minutes after addition of the Stop Solution.

13.Measurement

13.1 Adjust the microplate reader to zero using the Substrate blank.

ΔNote: If due to technical reasons that the microplate reader cannot be adjusted to zero using the blank, then subtract its absorbance value from all other absorbance values measured to obtain reliable results.

13.2 Measure the absorbance of all wells at 450 nm and record the absorbance values for each control and sample in the plate layout.

13.3 Biochromatic measurement using a reference wavelength of 620 nm is recommended.

13.4 Where applicable calculate the mean absorbance values for all duplicates.

13.5 Run validation criteria:

- For an assay to be considered valid, the following criteria must be met.
- If these criteria are not met, the test is not valid and must be repeated.

Controls	Absorbance value
Substrate Blank	< 0.1
Negative Control	< 0.2 and < Cut-off
Cut-off Control	0.15 – 1.3
Positive Control	> Cuf-off

14. Calculations

14.1 Calculate the average absorbance value for the Cut-off control and the samples.

$$\frac{\text{Sample (average) absorbance value} \times 10}{\text{Cut-off control (average value)}} = \text{Units}$$

$$\text{E.g. } \frac{1.591 \times 10}{0.43} = 37 \text{ Units}$$

	Units	Interpretation of results
Cut-off	10	
Positive	> 11	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11	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	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 based on 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.

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.

15. Typical Sample Values

PRECISION –

- The reproducibility of the kit was determined by comparing 24 replicates of 3 different samples in one assay (intra-assay) and by comparing 3 different samples assayed in 12 different runs (inter-assay).
- Acceptance Criterion: CV < 15 %

	Intra-Assay			Inter-Assay		
	1	2	3	1	2	3
n =	24	24	24	12	12	12
Mean (OD)	0.275	0.474	1.722	22.35	62.64	1.88
CV (%)	9.88	7.96	5.05	9.56	7.20	14.30

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 100% (95% confidence interval: 88.78% - 100%).

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 90% (95% confidence interval: 68.3% - 98.77%).

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.

CROSS REACTIVITY –

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters (rheumatoid factors and antibodies to *Candida albicans*) did not reveal evidence of false-positive results due to cross-reactions.

16. Notes

Technical Support

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