Instructions for Use

Legionella pneumophila IgG ELISA

Enzyme immunoassay for the qualitative determination of IgG class antibodies against Legionella pneumophila in human serum or plasma.

⏳	1 x 96 well plate
REF	ELS61256
📖	Read the documentation

For Research Use Only

CONTENTS

1. INTRODUCTION

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.

Legionella was first identified after an outbreak of pneumonia involving delegates of the 1976 American Legion Convention at a Philadelphia hotel.

The genus Legionella currently has at least 50 species comprising 70 distinct serogroups. One species of Legionella, L. pneumophila, is the aetiological agent of approximately 90 % of legionellosis cases, and serogroup 1 (Sg1) accounts for about 84 % of these cases.

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.

The likelihood of contracting Legionnaires' disease depends on the level of contamination in the water source, the susceptibility of the person exposed, and the intensity of exposure. Legionnaires' disease is characterized as an "opportunistic" disease that attacks individuals who have an underlying illness or a weakened immune system. Predisposing risks include increasing age, being male, heavy smoking, alcohol abuse, chronic lung disease, immunosuppressive therapy, cancer chemotherapy, organ or bone marrow transplant, and corticosteroid therapy.

Legionellosis can appear in two distinct clinical presentations: Legionella pneumonia (Legionnaires' disease) with an incubation period of approx. 2-10 days (may extend up to 16-20 days) and Pontiac fever (incubation period: normally 12-48 hours).

Legionella pneumonia (Legionnaires' disease) is a serious form of pneumonia that carries with it a case-fatality ratio of 10-15 %. Legionnaires' disease patients initially present with cough, fever and nonspecific symptoms including malaise, myalgia and headache. Some patients develop shaking chills, chest pain, diarrhea, delirium or other neurologic symptoms. Extra pulmonary involvement is rare.

Pontiac fever is a milder form of the disease without manifestations of pneumonia and presents as an influenza-like illness. Symptoms may include headache, chills, muscle aches, a dry cough and fever. It is usually self-limiting and typically does not require treatment. The attack rate is much higher than for Legionnaires' disease (up to 95 % of those exposed).

Species	Disease	Symptoms (e.g.)	Transmission route
Legionella pneumophila	Legionella pneumonia (Legionnaires' disease) Pontiac fever	Cough, fever and nonspecific symptoms (malaise, myalgia, headache). Some patients develop shaking chills, chest pain, diarrhea, delirium or other neurologic symptoms Influenza-like illness (headache, chills, muscle aches, a dry cough and fever) without manifestations of pneumonia	Inhalation of aerosols containing Legionella bacteria or micro-aspiration of ingested water contaminated with Legionella

The presence of pathogen or infection may be identified by

- Culture
- Urinary antigen detection
- PCR
- Serology: Detection of antibodies by ELISA

2. INTENDED USE

The Legionella pneumophila IgG ELISA is intended for the qualitative determination of IgG class antibodies against Legionella pneumophila 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.

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.

4. MATERIALS

4.1. Reagents supplied

- **Legionella pneumophila Coated Microplate (IgG):** 12 break-apart 8-well snap-off strips coated with Legionella pneumophila antigens; in resealable aluminium foil.
- **IgG Sample Diluent:** 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.
- **Stop Solution:** 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Buffer (20x conc.):** 2 bottles containing 25 ml each of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Legionella pneumophila anti-IgG Conjugate:** 1 bottle containing 20 ml of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap; < 5% NMP. *Note: NMP may damage an unborn child. Wear protective gloves and eye protection when handling TMB Substrate Solution. Get medical advice in case of exposure.*
- **Legionella pneumophila IgG Positive Control:** 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; red cap.
- **Legionella pneumophila IgG Cut-off Control:** 1 vial containing 3 ml control (human serum or plasma); coloured yellow; ready to use; green cap.
- **Legionella pneumophila IgG Negative Control:** 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; blue cap.

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 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
- 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. Coated Microplate

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 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 Diluent. Dispense 10 µl sample and 1 ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

8.1. Test Preparation

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 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 und 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.

8.2. Measurement

Adjust the ELISA microwell plate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA microwell plate 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 to be considered valid, 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]

Sample (mean) absorbance value x 10   =   [Units = U]
            Cut-off

Example: $\dfrac{1.591 \times 10}{0.43}$ =   37 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.

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: $\rightarrow$ suggests a current or very recent infection Rare: $\rightarrow$ persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: $\rightarrow$ may indicate a past infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

10.1. Precision

Intraassay	n	Mean (E)	CV (%)
#1	24	0.275	9.88
#2	24	0.474	7.96
#3	24	1.722	5.05

Interassay	n	Mean (U)	CV (%)
#1	12	22.35	9.56
#2	12	62.64	7.20
#3	12	1.88	14.30

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 100.0% (95% confidence interval: 88.78% - 100.0%).

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 90.0% (95% confidence interval: 68.3% - 98.77%).

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 test kits 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 samples.
- For research use only.
- 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 <u>anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive</u>.
- Do not interchange reagents or strips 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 samples and dispense reagents without splashing <u>accurately</u> into the wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

12.1. 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.

13. ORDERING INFORMATION

Prod. No.:   ELS61256   Legionella pneumophila IgG ELISA (96 Determinations)

BIBLIOGRAPHY

Bartram, Jamie; Chartier, Yves; Lee, John V.; Pond, Kathy; Surman-Lee, Susanne (Eds.) (2007): Legionella and the prevention of legionellosis. Geneva: WHO (14).

Darby, Jonathan; Buising, Kirsty (2008): Could it be Legionella? In *Australian Family Physician* 37 (10), pp. 812–815.

Fields, Barry S.; Benson, Robert F.; Besser, Richard E. (2002): Legionella and Legionnaires' Disease. 25 Years of Investigation. In *Clinical Microbiology Reviews* 15 (3), pp. 506–526. DOI: 10.1128/CMR.15.3.506-526.2002.

Joseph, C. A. (2004): Legionnaires' disease in Europe 2000-2002. In *Epidemiology and infection* 132 (3), pp. 417–424. DOI: 10.1017/S0950268804002018.

Marrie, Thomas J.; Hoffman, Paul (2006): Legionellosis. In Richard L. Guerrant, David H. Walker, Peter F. Weller (Eds.): Tropical infectious diseases. Principles, pathogens & practice. 2nd ed. Philadelphia: Churchill Livingstone, pp. 374–380.

Robert Koch Institut (RKI) (2013): Legionellose. In *RKI-Ratgeber für Ärzte*.

Steinert, Michael; Hentschel, Ute; Hacker, Jörg (2002): Legionella pneumophila: an aquatic microbe goes astray. In *FEMS microbiology reviews* 26 (2), pp. 149–162.

Stout, Janet E.; Yu, Victor L. (1997): Legionellosis. In *The New England Journal of Medicine* 337 (10), pp. 682–687. DOI: 10.1056/NEJM199709043371006.

Yu, Victor L.; Plouffe, Joseph F.; Pastoris, Maddalena Castellani; Stout, Janet E.; Schousboe, Mona; Widmer, Andreas et al. (2002): Distribution of Legionella species and serogroups isolated by culture in patients with sporadic community-acquired legionellosis: an international collaborative survey. In *The Journal of Infectious Diseases* 186 (1), pp. 127–128. DOI: 10.1086/341087.

Zuravleff, Jeffrey J.; Yu, Victor L.; Shonnard, John W.; Davis, Bridgett K.; Rihs, John D. (1983): Diagnosis of Legionnaires' disease. An update of laboratory methods with new emphasis on isolation by culture. In *JAMA* 250 (15), pp. 1981–1985.

ABBREVIATIONS

NMP	N-Methyl-2-pyrrolidone

SYMBOLS KEY

LOT	Lot Number
⧖	Expiration Date
⌇	Storage Temperature
REF	Catalogue Number
📖 i	Consult Instructions for Use
MTP	Microplate
CONJ	Conjugate
CONTROL -	Negative Control
CONTROL +	Positive Control
CUT OFF	Cut-off Control
DIL G	IgG Sample Diluent
SOLN STOP	Stop Solution
SUB TMB	TMB Substrate solution
WASH BUF 20x	Washing Buffer 20x concentrated
Σ n	Contains sufficient for "n" tests

SUMMARY OF TEST PROCEDURE

SCHEME OF THE ASSAY
Legionella pneumophila 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 °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)					