



User's Manual

Leishmania Antibody ELISA Kit



DEIA2425



96T



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

This kit is intended for the qualitative determination of IgG class antibodies against *Leishmania infantum* in human serum or plasma (citrate, heparin).

General Description

Leishmania are protozoa belonging to the family trypanosomatidae. The parasites exist in two forms: the promastigotes in the midgut of the vector insect, and the amastigotes within the phagolysosomes of macrophages in their mammalian hosts. In the macrophages they live as round, non-motile amastigotes (3-7 µm in diameter). The macrophages are ingested by the sandfly during blood-meal and the amastigotes are released into their stomach. Almost immediately the amastigotes transform into the motile, elongated (10-20 µm), flagellate promastigote form, which migrate to the alimentary tract of the fly and after multiplication move forward to the salivary glands of the insect. Leishmaniasis is a globally widespread group of parasitic diseases; the "type" is determined by the primary location of the macrophages that are infected. In humans four different forms of Leishmaniasis with a broad range of clinical manifestations are present; all can have devastating consequences. Leishmaniasis currently affects some 12 million people in 88 countries, all but 16 of which are in the developing world. It is estimated that 350 million people are exposed to the risk of infection by the different species of *Leishmania* parasite; the annual incidence of new cases is about 2 million (1-1.5 million cases of CL, 500,000 cases of VL). Visceral leishmaniasis (VL) is the most severe form of the disease, which, if untreated, has a mortality rate of almost 100%. Like many other tropical diseases, the leishmaniasis are related to economic development and man-made environmental changes, which increase exposure to the sandfly vector. The geographical distribution is limited by the distribution of the sandfly. AIDS and other immunosuppressive conditions increase the risk of *Leishmania* infected people developing visceral illness (VL). *Leishmania*/HIV co-infections are considered to be a real "emerging disease", especially in southwestern Europe, where 25-70% of adult VL cases are related to HIV infection, and 1.5-9.5% of AIDS cases suffer from newly acquired or reactivated VL. Intravenous drug users have been identified as the main population at risk.

Principles of Testing

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.

Reagents And Materials Provided

- 1. Microtiterplate:** 12 break-apart 8-well snap-off strips coated with *Leishmania infantum* antigens; in resealable aluminium foil.
- 2. 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 Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- 4. Washing Buffer (20× 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. Conjugate:** 1 bottle containing 20 mL of peroxidase Protein A in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- 6. TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), $< 0.1\%$; ready to use; yellow cap.
- 7. Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; $\leq 0.02\%$ (v/v) MIT.
- 8. Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; $\leq 0.02\%$ (v/v) MIT.
- 9. Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; $\leq 0.0015\%$ (v/v) CMIT/ MIT (3:1).

Materials Required But Not Supplied

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

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.

Specimen 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.

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.

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!

1. Microtiterplate

The break-apart snap-off strips are coated with *Leishmania infantum* 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.

2. Washing Buffer (20× 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.

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.

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 \pm 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.

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.

Calculation

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:

- a. Substrate Blank: Absorbance value < 0.100
- b. Negative Control: Absorbance value < 0.200 and < Cut-off
- c. Cut-off Control: Absorbance value 0.150 – 1.300
- d. Positive Control: Absorbance value > Cut-off

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

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

2. Results in Units

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

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

Interpretation Of Results

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

Precision

Intraassay	n	Mean (E)	CV (%)
#1	24	0.463	4.25
#2	24	1.245	5.95
#3	24	0.727	3.59
Interassay	n	Mean (CDU)	CV (%)
#1	12	27.34	4.94
#2	12	29.33	6.54
#3	12	1.23	14.19

Specificity

A cross reaction with antibodies against Chagas cannot be excluded

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.

Precautions

1. 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.
2. Only for in-vitro research use.
3. All materials of human or animal origin should be regarded and handled as potentially infectious.
4. 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.
5. Do not interchange reagents or Microtiterplates of different production lots.
6. No reagents of other manufacturers should be used along with reagents of this test kit.
7. Do not use reagents after expiry date stated on the label.
8. Use only clean pipette tips, dispensers, and lab ware.

9. Do not interchange screw caps of reagent vials to avoid cross-contamination.
10. Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
11. After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
12. To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
13. The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
14. For further internal quality control each laboratory should additionally use known samples.

