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User's Manual

Leishmania IgG ELISA Kit

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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 classic Leishmania IgG test is a qualitative and quantitative immunoassay for the detection of human IgG antibodies in serum or plasma directed against Leishmania.

General Description

Leishmaniasis is an infectious disease caused by trypanosomes of the genus Leishmania. This disease occurs predominantly in tropical and subtropical climate zones. Farm and domestic animals are primarily affected, however, the disease can also be transmitted to humans.

Leishmaniasis occurs worldwide with high incidences in Eastern Africa, South America, and Asia; cases have also been reported in the Mediterranean area. Annually, approx. 1.5 million cases of cutaneous and 0.5 million cases of visceral leishmaniasis are reported.

Dogs and rodents serve as the main reservoirs for Leishmania, but cats, horses, sheep, and cattle may also be afflicted. Sandflies (phlebotominae) or other moth flies (psychodidae) transmit the parasites to humans. The incubation period is variable, ranging from a few weeks up to several years.

Following transmission, the parasites have a particular affinity for macrophages of the host which they infect and in which they proliferate largely protected from an immune reaction. After destruction of infected macrophages, the released Leishmania can spread and infect other host cells.

Depending on the host's immune status, the various Leishmania species can induce different clinical manifestations: cutaneous, mucocutaneous, or visceral leishmaniasis. The cutaneous form presents generally with mild symptoms and heals spontaneously, but the visceral form can be lethal.

Cutaneous leishmaniasis (Baghdad boil, oriental sore) is frequently caused by L. tropica, L. major, or L. aethiopica. Proliferation of the parasites is mainly restricted to the site of infection. Following an erythematous rash, a non-painful ulcer can develop often accompanied by swollen local lymph nodes. In most cases, cutaneous leishmaniasis is a self-limiting disease although scarring can result.

Mucocutaneous leishmaniasis (uta, espundia) is caused by L. brasiliensis and affects skin and mucus membranes in the nasal region, the oral cavity of the pharyngeal region, and sometimes the genitals. The disease manifests with severe skin ulceration and tissue destruction. Infections with L. donovani and L. infantum may induce visceral leishmaniasis (kala-azar, black fever, dumdum fever). The disease manifests primarily with flu-like symptoms, swollen lymph nodes, and recurring fever accompanied by abdominal pain, nausea, vomiting, and diarrhea. The palms of hand, the soles of feet, and the mucus membranes are noticeably dark-colored. Depending on the organs involved, additional symptoms may occur.

Principles of Testing

The ELISA (Enzyme-Linked Immunosorbent Assay) is an immunoassay suited to the detection of antibodies. The reaction is based on the specific interaction of antibodies with their corresponding antigen. The test strips of the ELISA microtiter plate are coated with specific antigens of the pathogen of interest. If antibodies in a sample are present, they bind to the fixed antigen. A secondary antibody, which has been conjugated with the enzyme alkaline phosphatase, detects and binds to the antigen-antibody complex. The colorless

substrate p-nitrophenylphosphate is then converted into the colored product p-nitrophenol. The signal intensity of this reaction product is proportional to the concentration of antibody in the sample and is measured photometrically.

Reagents And Materials Provided

1. Break apart microtiter test strips each with eight antigen coated single wells, (altogether 96), 1 frame. The coating material is inactivated. 12

2. Standard serum (ready-to-use), Human serum in protein-containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface Antigen) and anti-HCV Ab; Preservative: <0.1% sodium azide; coloring: Amaranth O. 2 × 2 ml

3. Negative control serum (ready-to-use), Human serum in protein-containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface Antigen) and anti-HCV Ab; Preservative: <0.1% sodium azide; coloring: Lissamin Green V. 2 ml

4. Anti-human IgG conjugate (ready-to-use), Anti-human IgG polyclonal antibody, Conjugated to alkaline phosphatase, stabilized with protein stabilization solution; Preservative: <0.1% methylisothiazolone, <0.1% bromnitrodioxane. 13ml

5. Washing solution concentrate (sufficient for 1000ml), Sodium chloride solution with Tween 20 and 30mM Tris-HCl, pH 7.4; Preservative: <0.1% sodium azide. 33.3ml

6. Dilution buffer (ready-to-use), Protein-containing phosphate buffer with Tween 20; Preservative: <0.1% sodium azide; coloring: 0.01g/l Bromphenol blue. 2 × 50ml

7. Stopping solution (ready-to-use), <0.1N sodium hydroxide, 40mM EDTA. 15ml

8. Substrate (ready-to-use), Para-nitrophenylphosphate in solvent-free buffer; Preservative: <0.1% sodium azide. 13ml

Materials Required But Not Supplied

1. Common laboratory equipment
2. Photometer for microtiter plates with filter, wavelength 405nm, recommended reference wavelength 620nm-690nm (e.g., 650nm)
3. Microtiter plate washer
4. Incubator 37°C
5. Moist chamber
6. Distilled water
7. Optional: ELISA control

Storage

Reagent	Storage	Stability
Microtiter strips (coated with antigen)	Unopened After opening at 2-8°C in closed aluminum bag with desiccant	See expiry date Minimum shelf-life 4 weeks
Control sera / Standard sera	Unopened / after opening at 2-8°C	See expiry date
Conjugate	Unopened / after opening at 2-8°C	See expiry date
Dilution buffer	Unopened / after opening at 2-8°C	See expiry date
Washing solution	Unopened / after opening at 2-8°C Working dilution at 2-8°C Working dilution at room temperature	See expiry date 2 weeks 1 week
Substrate	Unopened / after opening at 2-8°C	See expiry date
Stopping solution	Unopened / after opening at 2-8°C	See expiry date

Specimen Collection And Preparation

1. Sample Preparation and Storage

Lipaemic, hemolytic or icteric samples (serum or plasma) should only be tested with caution. Obviously contaminated samples should not be tested. Serum or plasma (EDTA, citrate, heparin) collected according to standard laboratory methods are suitable samples. Samples must not be thermally inactivated.

2. Dilution of Samples

Before running the test, samples (V_1) must be diluted in dilution buffer (V_2) as follows:

$V_1 + V_2 = 1:100$	add	10ul	sample
	each to	1000ul	dilution buffer

After dilution and before pipetting into the microtiter plate, the samples must be mixed thoroughly to prepare a homogenous solution.

3. Sample Storage

Samples should not be stored more than 7 days at 2-8°C. Extended storage is possible at ≤-20°C. Avoid repeated freezing and thawing of samples. Diluted samples can be stored at 2-8°C for one week.

Reagent Preparation

Bring all reagents to room temperature before use.

1. Microtiter Test Strips

The microtiter test strips labeled with abbreviations for pathogen and immunoglobulin class are packed with a desiccant in an aluminum bag. To open the aluminum bag of the microtiter plate, please cut off the top of the marked side only in order to guarantee proper resealing. Take unrequired wells out of the frame and put them back into the aluminum bag. Close bag carefully to ensure airtight conditions. Do not use strips if the aluminum bag is damaged or if the bag with remaining strips and desiccant was not properly resealed.

2. Negative Control Sera / Standard Sera (ready-to-use)

Negative control and standard sera are ready-to-use and must not be diluted any further. For each test run (independent of the number of microtiter test strips to be used) negative control and standard sera must be included. Standard sera should be set up in duplicate. Do not treat negative control and standard sera with

Rf-absorbent.

3. Anti-human IgG AP-Conjugate (ready-to-use)

The required conjugate concentration (i.e., +, ++, +++) is indicated on the quality control certificate. Please refer also to the specification on the label. Avoid contamination.

4. Washing Solution (Concentrate)

Dilute washing buffer concentrate (V_1) 1:30 with distilled H2O to a final volume of V_2.

Example:

buffer concentrate (V_1)	final volume (V_2)
33.3 ml	1000 ml
1 ml	30 ml

5. Dilution Buffer for Samples (ready-to-use)

6 Substrate (ready-to-use)

Substrate in unopened bottle may have a slight yellow color which does not reduce the quality of the product! Avoid contamination.

7. Stopping Solution (ready-to-use)

Assay Procedure

1. Place the required number of wells in the frame and prepare a protocol sheet.
2. Add each 100ul of diluted sample or ready-to-use negative control / standard sera into the appropriate wells of microtiter test strips. Spare one well for substrate blank, e.g.:

   A1 Substrate blank

   B1 Negative control

   C1 Standard serum

   D1 Standard serum

   E1 Sample 1 . . .

   F1 Sample 2 . . .
3. Sample incubation for 60 minutes (+/- 5 min) at 37°C (+/- 1°C) in moist chamber.
4. After incubation wash all wells with washing solution (by automated washer or manually):

   aspirate or shake out the incubation solution

   fill each well with 300ul washing solution

   aspirate or shake out the washing solution

   repeat the washing procedure 3 times (altogether 4 times!)

   dry by tapping the microtiter plate on a paper towel
5. Addition of conjugate: Add 100ul of the ready-to-use IgG conjugate to the appropriate wells (except substrate blank).
6. Conjugate incubation for 30 minutes (+/- 1 min) at 37°C (+/- 1°C) in moist chamber.

7. After incubation wash all wells with washing solution (see above).
8. Addition of substrate: Add 100ul of ready-to-use substrate solution to each well (including well for substrate blank!)
9. Substrate incubation for 30 minutes (+/- 1 min) at 37°C (+/- 1°C) in moist chamber. Ensure incubation is in the dark.
10. Stopping the reaction: Add 100ul of stopping solution to each well, shake microtiter plate gently to mix.
11. Read extinction: Read optical density (OD) within 60 minutes at 405nm against substrate blank, reference wavelength between 620nm and 690nm (e.g. 650nm).

Quality Control

For the periodic verification of the test method and in order to fulfill the requirements of laboratory internal quality management systems, we recommend using ELISA controls determine precision and accuracy of ELISA test runs. The use of ELISA controls is described in specific instruction manuals.

Calculation

1. ELISA Leishmania IgG

The mathematical curve fitting for antibody quantification with ELISA immunoassays is based on the 4-parameter logistic (4 PL) function.

$$Activity\ (U/ml) = e^{C-\frac{1}{B}\ln\left(\frac{D-A}{OD(Patient)*F-A}-1\right)}$$

The 4 parameters A, B, C, and D are representative for the exact shape of the standard curve:

Parameter A: Lower asymptote (OD)

Parameter B: Slope of the curve

Parameter C: Inflection point

Parameter D: Upper asymptote (OD)

CD establishes a lot-specific 4 PL standard curve for each ELISA immunoassay in multiple test runs under optimal test conditions. The four parameters are indicated on the quality control certificate of each individual ELISA test.

For the adaptation of the test level to the given 4 PL standard curve, the correction factor F is calculated by dividing the standard reference OD value indicated on the quality control certificate with the measured, and consequently test run-specific, standard OD value.

$$F = \frac{STD\ reference\ OD\ value}{measured\ \ STD\ OD\ value}$$

By multiplying the OD values obtained from samples with the correction factor F, the level of each individual test run is adjusted to the given 4 PL standard curve. Thereby, interassay deviations are compensated for

and antibody activities can be directly evaluated from the 4 PL standard curve.

After subtraction of the substrate blank from all measured OD values and calculation of the mean OD value of the standard serum (STD), tested in duplicate, a range of possibilities are available for the evaluation of antibody activities from the optical measurement signals (OD) of samples. They are described in separate manuals.

Borderline Range

The borderline range of the ELISA Leishmania IgG test is specified on the quality control certificate and indicates the range of borderline test results. Values below this range indicate a negative result; values above the borderline range indicate a positive result.

Criteria of Validity

a. The substrate blank must be <0.25 OD.

b. The negative control must be negative.

c. For use of quantitative SERION ELISA classic tests, the mean OD value (after subtraction of the substrate blank!) of the standard serum must be within the validity range which is given on the lot-specific quality control certificate.

d. For use of qualitative ELISA tests, the OD value of the positive control and the mean OD value of the cut-off serum must be within the validity ranges which are given on the lot-specific quality control certificate of the kit (after subtraction of the substrate blank!)

e. The variation of OD values of the standard serum or cut-off serum must not be higher than 20%.

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

Precautions

Optimum results can only be achieved if the instructions are strictly followed. Only use ELISA reagents when using ELISA immunoassays. The components must not be exchanged for reagents of other manufacturers. Standard and control sera of ELISA immunoassays are defined exclusively for the test kit to be used and must not be used in other lots.

Each ELISA test contains a ready-to-use sample dilution buffer. In some cases the use of special dilution buffers is necessary to guarantee consistent quality and reliable results.

There are three different conjugate concentrations for each immunoglobulin class (IgA, IgG, IgM) indicated on the label as + (low), ++ (medium), and +++ (high). Conjugates with the same concentration and of the same immunoglobulin class are interchangeable and can be used for other ELISA immunoassays irrespective of the lot and the test. Dilution or alteration of the reagents may result in a loss of sensitivity. Use aseptic techniques when removing aliquots from the reagent tubes to avoid contamination.

Reproducibility of test results is dependent on thorough mixing of the reagents. Agitate the vials containing control sera before use and also all samples after dilution (e.g., by using a vortex mixer).

Be sure to pipette carefully and comply with the given incubation times and temperatures.

Significant time differences between pipetting the first and last well of the microtiter plate when dispensing samples and control sera, conjugate or substrate can result in different preincubation times, which may influence the precision and reproducibility of the results. Avoid exposure of reagents to strong light during storage and incubation.

Adequate washing avoids test unspecificities. Therefore, the washing procedure should be carried out carefully. All of the flat bottom wells should be filled with equal volumes of washing buffer. At the end of the procedure ensure that the wells are free of all washing buffer in order to avoid uncontrolled dilution effects. Avoid foaming!

Reagents must be tightly closed after use to avoid evaporation and contamination. Take care not to mix up the caps of the bottles and/or vials.

The ELISA immunoassay is only valid if the lot-specific validation criteria on the quality control certificate are fulfilled.

Statements of Warning

The ELISA is designed for use by qualified personnel who are familiar with good laboratory practice. All kit reagents and human samples should be handled carefully using established good laboratory practice.

a. This kit contains human blood components. Although all control- and cut-off sera have been tested and found negative for anti-HIV Ab, HBs-Ag (Hepatitis B Virus surface Antigen) and anti-HCV Ab, they should be considered potentially infectious.

b. Do not pipette by mouth.

c. Do not smoke, eat, or drink in areas in which samples or kit reagents are handled.

d. Wear disposable gloves, laboratory coat, and safety glasses while handling kit reagents or samples. Wash hands thoroughly afterwards.

e. Samples and other potentially infectious material should be decontaminated after use.

f. Reagents should be stored safely and be inaccessible to unauthorized access, e.g. children.

Limitations

The limits of quantification are specified on the quality control certificate of the ELISA Leishmania IgG. The linearity of dilution within this range has been demonstrated in comprehensive evaluation studies. If a sample shows a test result above the upper limit of quantification, the sample may be tested at a higher dilution. The resulting antibody activity must then be multiplied by the additional dilution factor.