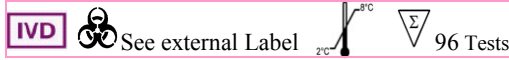


**AccuDiag™  
 Leishmania  
 ELISA Kit**

REF 8203-35



<b>Test</b>	<b>Leishmania ELISA</b>
<b>Method</b>	<b>Enzyme Linked Immunosorbent Assay</b>
<b>Principle</b>	<b>Sandwich Complex</b>
<b>Detection Range</b>	<b>Qualitative Positive ; Negative Control</b>
<b>Sample</b>	<b>10 µL serum</b>
<b>Total Time</b>	<b>~ 25 min.</b>
<b>Shelf Life</b>	<b>12 Months from the manufacturing date</b>
<b>Specificity</b>	<b>84%</b>
<b>Sensitivity</b>	<b>97%</b>

**INTENDED USE**

The **Leishmania ELISA Kit** is for the screening of serum antibodies, primarily IgG, for visceral *Leishmania* using the ELISA technique.

**SUMMARY AND EXPLANATION**

Visceral Leishmaniasis (VL) is a severe disease with high mortality, caused by parasite members of the *L. donovani* complex (1,2). The vector for transmission is the sand fly, whose carriers of infection are typically dogs (3-6). It is a disease endemic to many countries and is a serious problem in many developing nations, particularly with the increasing urbanization of populations (7). High incidence is encountered in parts of Latin America, East Africa, Middle East, India and China. It is endemic to countries bordering the Mediterranean such as Italy, Southern France, Spain, Portugal, and Northern Africa. In Southern Europe, VL has become the leading opportunistic infection in AIDS patients (8-15).

Diagnosis of acute VL is often attempted by aspiration of bone marrow for direct parasite identification. The procedure is invasive, painful, dangerous and has a low success rate due to the inability to always isolate parasites from the tissue. Alternatively, serodiagnosis is widely utilized since anti-leishmanial antibody titers are typically high during the acute disease phase. ELISA is the preferred laboratory test for serodiagnosis of VL, although indirect immunofluorescent antibody tests (IFAT) and direct agglutination tests (DAT), using whole parasites, are still widely used in conjunction with ELISA or alone (16-18).

**TEST PRINCIPLE**

During the first incubation, the antibodies in the patient's serum bind to the antigens in the test well. The next incubation allows the antigen-antibody complex to bind to an enzyme complex. After washing the wells to remove unbound enzyme, a chromogen is added that develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction and turns the blue color to yellow.

**SPECIMEN COLLECTION AND PREPARATION**

Coagulate blood and remove serum. Freeze sample at -20 °C or lower if not used immediately.  
 Do not heat inactivate serum.  
 Avoid repeated freezing and thawing of samples.

**MATERIALS AND COMPONENTS**

**Materials provided with the test kits**

- Plate:** Microwells containing Leishmania antigens - 96 test wells in a test strip holder.
- Enzyme Conjugate:** One (1) bottle containing 11 ml of anti-human Ig-Peroxidase (HRP) in a stabilizing buffer with Thimerosal.
- Positive Control:** One (1) vial containing 1 ml of diluted Leishmania-positive human sera in buffer with Thimerosal.
- Negative Control:** One (1) vial containing 1 ml of diluted Leishmania-negative human sera in buffer with Thimerosal.
- TMB Substrate Solution:** One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).
- Wash Concentrate 20X:** One (1) bottle containing 25 ml of concentrated buffer and surfactant.
- Dilution Buffer:** Two (2) bottles containing 30 ml of buffered protein solution.
- Stop Solution:** One (1) bottle containing 11 ml of 1 M phosphoric acid.

**Materials required but not provided**

- Pipettes
- Squeeze bottle for washing strips
- DI Water
- ELISA plate reader with a 450 nm and a 620-650 nm filter (optional if results are read visually)
- Tubes for serum dilutions

**Preparation**

Wash Buffer - Remove cap and add contents of bottle to 475 ml of reagent grade water. Place diluted wash buffer into a squeeze bottle.

**Note:** Washings consist of filling to the top of each well, shaking out the contents and refilling.  
 Avoid generating bubbles in the wells during the washing steps.

Test samples: Make a 1:40 dilution of patients' sera using the dilution buffer.

**ASSAY PROCEDURE**

- Break off number of wells needed (two for controls plus number of samples) and place in strip holder.
- Add 100 µl of negative control to well #1, 100 µl of positive control to well #2, and 100 µl of the diluted (1:40) test samples to the remaining wells.  
 Note: Negative and positive controls are supplied as prediluted. Do not dilute further.
- Incubate at room temperature (15 °C to 25 °C) for 10 minutes.
- Shake out contents and wash 3 times with diluted wash buffer.\*
- Add 2 drops of enzyme conjugate to each well.
- Incubate at room temperature for 10 minutes.
- Shake out contents and wash 3 times with wash buffer.
- Add 2 drops of Chromogen to every well.
- Incubate at room temperature for 5 minutes.
- Add 2 drops of stop solution. Mix wells by tapping plate.
- Zero ELISA reader on air, read wells at 450 nm with a reference filter at 620-650 nm or read results visually.

\* Washings consist of using the diluted wash buffer to fill to the top of each well, shaking out the contents and refilling the wells for a total of 3 times.

Avoid generating bubbles in the wells during the washing steps.

Controls must be included each time the kit is run.

**\*CAUTION! WHEN USING AN AUTOMATED OR SEMI-AUTOMATED WASHING SYSTEM THE FOLLOWING MUST BE FOLLOWED. FAILURE TO DO SO WILL RESULT IN INADEQUATE WASHING OF THE WELLS AND MAY LEAD TO FALSE POSITIVE RESULTS!**

**Washing Procedure for Auto and Semi-Automated Washers**

- Perform five (5) washes per step instead of three
- Set machine to "soak" for one minute between each step
- After each set of washings, slap wells against an absorbent towel.

**RESULTS**

**Spectrophotometer:**

Zero ELISA reader on air. Read all wells using a bichromatic reading with filters at 450 nm and 620-650 nm.

**Positive** - Absorbance reading greater or equal to 0.2 OD units.

**Negative** - Absorbance reading less than 0.2 OD units.

**Visual**

A sample should be interpreted as positive if the degree of color development is obvious and significant.

**Troubleshooting**

**Problem:** Negative control has substantial color development.

**Correction:** Inadequate washings. Rerun test with more vigorous washings.

**QUALITY CONTROL**

The use of a positive and negative control allows easy validation of kit stability. For a valid test, the positive control must be over 0.3 OD units and the negative control must be under 0.2 units. Should the values fall outside these ranges, the kit should not be used.

**PERFORMANCE CHARACTERISTICS**

		Reference Method *	
		+	-
Diagnostic Automation, Inc.	+	30	10
	-	1	53

**Sensitivity:** 30/31 = 97%

**Specificity:** 53/63 = 84%

**LIMITATIONS OF PROCEDURE**

Serological results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.

Although no specific cross reactions have been recorded to date, reactions by similar organisms cannot be ruled out.

**PRECAUTIONS**

1. Controls and dilution buffer are casein based buffer and will appear cloudy. In addition, a gelatinous plug may develop at the bottom of the vial. This is normal and does not affect the assay.
2. Wash concentrate may show crystallization upon storage at 4 °C. Crystallization will disappear after diluting to working strength.
3. Do not use serum that may have supported microbial growth, or is cloudy due to high lipid content. Samples high in lipids should be clarified before use.
4. Do not add azides to the samples or any of the reagents.
5. Controls and some reagents contain Thimerosal as a preservative.

6. Treat all sera as if capable of being infectious.
7. The controls has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV by required test methods. Since no test can offer complete assurance that infectious agents are not present, this product should be used under appropriate safety conditions that would be used for any potentially infectious agent.

**STORAGE**

**Reagents, strips and bottled components:**



Store between 2 – 8 °C.

Squeeze bottle containing diluted wash buffer may be stored at room temperature.

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<p><b>ISO 13485</b> <b>ISO 9001</b></p>  <p> <b>Diagnostic Automation/Cortez Diagnostics, Inc.</b>  <b>21250 Califa Street, Suite 102 and 116,</b>  <b>Woodland Hills, California 91367 USA</b></p>	
<b>Date Adopted</b>	<b>2016-11-18</b>
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EC REP	<b>CEpartner4U, Esdoornlaan 13,</b> <b>3951DB Maarn. The Netherlands.</b> <a href="http://www.cepartner4u.eu">www.cepartner4u.eu</a>
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