



AccuDiag™ Leishmania IgG ELISA Kit

REF 8203

PIC PB8203ZT1

IVD See External Label 2°C 8°C 96 Tests

Leishmania IgG ELISA	
Principle	Indirect ELISA: Antigen-Coated Plate
Detection	Qualitative
Sample	10 µL serum/plasma
Incubation Time	25 minutes
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

For the screening of serum or plasma antibodies, primarily IgG, for visceral *Leishmania* using the ELISA technique.

ASSAY PRINCIPLE

During the first incubation, the antibodies in the sera or plasma 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 & HANDLING

Serum or plasma (collected with heparin, EDTA, or citrate) should be stored at 2°C - 8°C if it is to be analyzed within 5 days. Samples may be held for extended storage at -20°C or lower for 1 year. Do not heat inactivate samples. Avoid repeated freezing and thawing of samples.

REAGENTS

Item	Description
Microtiterwells	Microwells containing Leishmania antigens – 96 test wells in a test strip holder.
Enzyme Conjugate	One (1) bottle containing 11 ml of anti-human IgG 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.
Chromogen TMB	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.

1. Micropipettes
2. Squeeze bottle for washing strips
3. DI Water
4. ELISA plate reader with a 450/620-650 nm filter (optionally, results can be read visually).
5. Tubes for serum dilutions
6. Timer

REAGENT 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

1. Break off number of wells needed (two for controls plus number of samples) and place in strip holder.
2. Add 100 µL of negative control to well #1,



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I M M U N O D I A G N O S T I C S

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.

3. Incubate at room temperature (15°C to 25°C) for **10 minutes**.
4. Shake out contents and wash 3 times with diluted wash buffer.*
5. Add **2 drops (100 µL)** of Enzyme Conjugate to each well.
6. Incubate at room temperature for **10 minutes**.
7. Shake out contents and wash 3 times with wash buffer.
8. Add **2 drops (100 µL)** of Chromogen to each well.
9. Incubate at room temperature for **5 minutes**.
10. Add **2 drops (100 µL)** of Stop Solution. Mix wells by tapping plate.
11. 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. If using automated washers: add 1 minute dwell time between washings and increase the number of washes from three to five. Avoid generating bubbles in the wells during the washing steps. Controls must be included each time the kit is run.

RESULTS

Visually:

Look at each well against a white background (e.g. paper towel) and record as clear or+,++ or+++ reaction.

ELISA Reader:

Zero reader on air. Set for bichromatic readings at 450/620-650 nm.

TROUBLESHOOTING

Problem: Negative control has substantial color development.

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

INTERPRETATION OF RESULTS

Spectrophotometer:

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

Negative: < 0.2 OD

Gray zone: 0.2 – 0.4 OD

Positive: > 0.2 OD

Interpretation of Results - Visual

Compare results to the controls.

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

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 0.20 OD units.

Should the values fall outside these ranges, the kit should not be used.

WARNINGS AND 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 CONDITIONS

Reagents, strips, and bottled components: Store between 2°C – 8°C. Squeeze bottle containing diluted wash buffer may be stored at room temperature.

MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

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Quality
Management for
Medical Devices
CERTIFIED

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