

## Human Entamoeba histolytica IgG (EH-Ab) ELISA Kit

**Catalog No.:** abx055727

**Range:** Qualitative

**Sensitivity:** Qualitative

**Storage:** Store at 2-8°C for 6 months.

**Application:** For qualitative detection of EH-Ab in Human Serum, Plasma, Tissue Homogenates and other biological fluids.

**Introduction:** Entamoeba histolytica is an anaerobic parasitic amoebozoan, part of the genus Entamoeba. Predominantly infecting humans and other primates causing amoebiasis, E. histolytica is estimated to infect about 50 million people worldwide. Previously, it was thought that 10% of the world population was infected, but these figures predate the recognition that at least 90% of these infections were due to a second species, E. dispar. Mammals such as dogs and cats can become infected transiently, but are not thought to contribute significantly to transmission. The active (trophozoite) stage exists only in the host and in fresh loose feces; cysts survive outside the host in water, in soils, and on foods, especially under moist conditions on the latter. The cysts are readily killed by heat and by freezing temperatures, and survive for only a few months outside of the host. When cysts are swallowed they cause infections by excysting (releasing the trophozoite stage) in the digestive tract. E. histolytica, as its name suggests (histo-lytic = tissue destroying), is pathogenic; infection can be asymptomatic or can lead to amoebic dysentery or amoebic liver abscess. Symptoms can include fulminating dysentery, bloody diarrhea, weight loss, fatigue, abdominal pain, and amoeboma. The amoeba can actually 'bore' into the intestinal wall, causing lesions and intestinal symptoms, and it may reach the blood stream. From there, it can reach different vital organs of the human body, usually the liver, but sometimes the lungs, brain, spleen, etc. A common outcome of this invasion of tissues is a liver abscess, which can be fatal if untreated. Ingested red blood cells are sometimes seen in the amoeba cell cytoplasm.

### Principle of the Assay

A 96 well plate has been pre-coated with an antigen specific to EH-Ab. Controls or test samples are added to the appropriate wells and incubated. Free components are washed away with wash buffer. HRP conjugated detection reagent is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue colour product that changes to yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the EH-Ab amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and the presence of EH-Ab can be determined.

### Kit components

1. One pre-coated 96 well plate
2. Positive Control: 0.5 ml
3. Negative Control: 0.5 ml
4. Wash buffer (30×): 20 ml. Dilution: 1:30
5. Sample diluent buffer: 6 ml
6. HRP Conjugate Reagent (RTU): 6 ml
7. Stop solution: 6 ml
8. TMB substrate A: 6 ml
9. TMB substrate B: 6 ml
10. Plate sealer: 2
11. Hermetic bag: 1

### Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. High-precision pipette and sterile pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare standard/sample dilutions
7. Deionized or distilled water
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

## Protocol

### A. Preparation of sample and reagents

#### 1. Sample

Isolate the test samples soon after collecting and analyze immediately at 1:5 dilution (within 2 hours) or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using citrate or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20°C.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

#### Note:

- » Coagulate blood samples completely, centrifuge, and avoid hemolysis and precipitant.
- » NaN<sub>3</sub> cannot be used as test sample preservative, since it inhibits HRP.
- » Store samples undiluted. Once ready to analyze, thaw samples and dilute 1:5.

#### 2. Wash buffer

Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

### B. Assay Procedure

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to plot a standard curve for each test.

1. Set positive/negative, test sample and control (zero) wells on the pre-coated plate respectively and record their positions.
2. Aliquot 50 µl of the negative and positive controls into the set wells. Leave one well as the control (zero) blank well.
3. Aliquot 50 µl of appropriately diluted sample into the test sample wells. Samples should be diluted 1:5. Add the solution at the bottom without touching the side walls of the well. Shake the plate mildly to mix the contents.
4. Seal the plate with a cover and incubate at 37°C for 30 min.
5. Remove the cover and discard the plate contents by tapping the plate on absorbent filter papers or other absorbent material.
6. Wash the plate 5 times with wash buffer. Do not let the wells completely dry at any time.

**Manual Washing:** Discard the solution without touching the side walls. Tap the plate on absorbent paper or other absorbent material. Fill each well completely with wash buffer and incubate on an ELISA shaker for 2 min. Discard the contents and tap the plate on absorbent filter papers or other absorbent material. Repeat this procedure five times.

**Automated Washing:** Discard the solution in all wells and wash the plate five times with Wash buffer (overfilling the wells with the buffer). After the final wash invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the

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washer be set for a soaking time of 1 min.

8. Add 50 µl of HRP conjugate reagent into each well (except control well). Add the solution at the bottom of each well without touching the side wall.
9. Seal the plate with a cover and incubate at 37°C for 30 min.
10. Remove the cover and repeat the aspiration/wash process 5 times as explained in step 7.
11. Aliquot 50 µl of TMB Substrate A into each well and 50 µl of TMB Substrate B. Vortex the plate gently on an ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds). Cover the plate and incubate at 37°C for 15 min. Avoid exposure to light.
12. Add 50 µl of Stop solution into each well. There should be a color change to yellow. Gently tap the plate to ensure thorough mixing.
13. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

### C. Analysis

#### 1. Calculations:

Mean Absorbance of PC = (O.D. of PC1 + O.D. of PC2) / 2 should be  $\geq 1.00$ .

Mean Absorbance of NC = (O.D. of NC1 + O.D. of NC2) / 2 should be  $\leq 0.10$ .

CUT OFF value = NC + 0.15

#### 2. Interpretation of results:

If the PC value is  $\geq 1.00$ , and NC value is  $\leq 0.10$ , the test is valid, otherwise, the test is invalid.

#### Samples:

If O.D. of samples < CUT OFF, the test samples are considered negative.

If O.D. of samples  $\geq$  CUT OFF, the test samples are considered positive.

### D. Precautions

1. Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
2. Avoid foaming or bubbles when mixing or reconstituting components.
3. Wash buffer may crystallize and separate. If this happens, please heat the tube to dissolve.
4. It is recommended measuring each controls and samples in duplicate or triplicate.
5. Do NOT let the plate dry out completely as this will inactivate the biological material on the plate.
6. Ensure plates are properly sealed or covered during incubation steps.
7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
8. Do not reuse pipette tips and tubes to avoid cross contamination.
9. Do not use expired components or components from a different kit.
10. The TMB Substrate solution is easily contaminated; work under sterile conditions when handling the TMB substrate solution. TMB Substrate solution should also be protected from light. Equilibrate the TMB substrate at room temperature prior to use. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.