Version 10-04/24

User's Manual

Entamoeba Ag ELISA Kit

REF **DEIA2309PY-1**

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 Entamoeba ELISA is an enzyme-linked immunosorbent assay (ELISA) for the qualitative identification of Entamoeba histolytica and Entamoeba dispar in human stool samples. For Research Use Only. Not for use in diagnostic procedures.

Principles of Testing

The Entamoeba ELISA uses specific antibodies in a sandwich-type method. The well surface of the microwell plate is coated with specific antibodies to the antigens of Entamoeba histolytica and Entamoeba dispar. A pipette is used to place a suspension of the stool sample to be examined as well as control samples into the well of the microwell plate together with biotinylated anti Entamoeba antibodies (Conjugate 1) for incubation at room temperature (20 - 25 °C). After a wash step, streptavidin poly-peroxidase conjugate (Conjugate 2) is added, and it is incubated again at room temperature (20 - 25 °C). With the presence of Entamoeba antigens in a sample, immobilized antibodies, the Entamoeba antigen, and the conjugated antibody form a sandwich complex. Another wash step removes the unattached streptavidin poly-peroxidase conjugate. After adding the substrate, the attached enzyme changes the color of the previously colorless solution in the wells of the microwell plate to blue if the test is positive. Addition of a stop reagent changes the color from blue to yellow. The extinction is proportional to the concentration of Entamoeba antigens in the sample.

Reagents And Materials Provided

1. Plate 1: 96-well microwell plate, 12 microwell strips (which can be divided) in strip holder, coated with specific monoclonal antibodies (mouse) to Entamoeba histolytica and Entamoeba dispar

2. Diluent 1: 100 ml Sample dilution buffer, protein-buffered NaCl solution; ready for use; blue colored

3. Wash Buffer: 100 ml Wash buffer, phosphate-buffered NaCI solution (concentrated 10 fold); contains 0.1 % thimerosal

4. Positive Control: 2 ml Inactivated Entamoeba antigen; ready for use

5. Negative Control: 2 ml Negative control (sample dilution buffer); ready for use

6. Conjugate 1: 13 ml Biotin conjugated antibodies (mouse) to Entamoeba antigens in stabilized protein solution; ready for use, green colored

7. Conjugate 2: 13 ml Streptavidin poly-peroxidase conjugate in stabilized protein solution; ready for use; orange colored

8. Substrate: 13 ml Hydrogen peroxide/TMB; ready for use

9. Stop: 12 ml Stop reagent; 1 N sulfuric acid; ready for use

10. Plate Sealer 2

Materials Required But Not Supplied

1. Distilled or deionized water

2. Precision micropipettes and standard laboratory pipettes
3. Graduated cylinder (1000 ml)
4. Clean glass or plastic tubes for the dilution of the samples
5. Timer
6. Microplate washer or multichannel pipette (300 µl)
7. Microplate reader (450 nm, reference filter 620-650 nm)
8. Filter paper (laboratory towels)
9. Vortex mixer (optional)

Storage

All reagents must be stored at 2 – 8 °C and can be used until the date printed on the label.

Providing the 1× working wash buffer is stored at 2 – 8 °C, it can be used for a maximum of 4 weeks. Microbial contamination must be prevented. After the expiry date, the quality guarantee is no longer valid.

The aluminum bag must be opened with scissors in such a way that the clip seal is not torn off.

Any microwell strips which are not required must be returned to the aluminum bag and immediately stored at 2 – 8 °C.

The colorless substrate must be protected from direct light to prevent it from decomposing or turning blue due to auto-oxidation. Once the substrate has turned blue, it must not be used.

Specimen Collection And Preparation

Until it is used, store the test material at 2 - 8 °C. If the material cannot be used for a test within three days, we recommend storage at –20 °C or colder. Avoid repeated freeze-thaw cycles.

After diluting a stool sample in sample dilution buffer 1:11, it can be stored at 2 - 8 °C for use within seven days (Table. 1).

Table. 1: Sample storage

Undiluted stool specimen		Diluted specimen
2 - 8 °C	≤ - 20 °C	2 - 8 °C
≤ 3 days	> 3 days	≤ 7 days

Stool samples and rectal smears should not be collected in transport containers which contain transport media with preservatives, animal sera, metal ions, oxidizing agents, or detergents since these may interfere with the Entamoeba Test. If rectal smears are used, make sure that the volume of stool material is sufficient (approx. 100 mg) for the test.

PREPARATION OF SAMPLES

Fill a labelled test tube with 1 ml sample dilution buffer Diluent 1. Use a disposable pipette to aspirate a sample of thin stool (approx. 100 µl) to just above the second bulge and add this to buffer in the test tube to make a suspension.

To suspend a solid stool sample, use an equivalent amount (approx. 50 - 100 mg) of the sample, handling it

with a spatula or disposable inoculation loop. Homogenize the stool suspension by aspiration into and ejection from a disposable pipette or, alternatively, blend in a Vortex mixer. Let the suspension stand a short period of time for the coarse stool particles to settle. This clarified supernatant of the stool suspension can be used directly in the test. If the test procedure is carried out in an automated ELISA system, the supernatant must be particle-free. In this case, it is recommended to centrifuge the sample at 2,500 G for 5 minutes.

Reagent Preparation

Wash Buffer

Mix 1 part wash buffer concentrate with 9 parts distilled water. Any crystals present in the concentrate must be dissolved beforehand by warming in a water bath at 37 °C.

Assay Procedure

All reagents and the microwell Plate must be brought to room temperature (20–25 °C) before use.

The microwell strips must not be removed from the aluminum bag until they have reached room temperature. The reagents must be thoroughly mixed immediately before use. After use, the microwell strips (placed in sealed bags) and the reagents must be stored at 2 - 8 °C. Once used, the microwell strips must not be used again. Reagents and microwell strips must not be used if the packaging is damaged or the vials are leaking.

To avoid cross contamination, the samples must be prevented from coming into direct contact with the kit components. The test must not be carried out in direct sunlight. It is recommended to cover the microwell plate or seal it with plastic wrap to prevent evaporation losses.

1. First incubation

After inserting a sufficient number of wells in the strip holder, add 100 µl of the positive control, the negative control, or the stool sample suspension to the wells. Subsequently add 100 µl of the biotin-conjugated antibody Conjugate 1 and blend (by tapping lightly on the side of the plate); then incubate for 30 minutes at room temperature (20 - 25 °C).

2. Washing

Careful washing is important to achieve the correct results and should proceed strictly as instructed. The incubated substance in the wells must be emptied into a waste container and discarded in accordance with local regulations. After, tap the plate onto absorbent paper to remove residual moisture. Then, wash the plate five times using 300 µl 1× working wash buffer each time. Make sure that the wells are emptied completely by tapping them after each wash on a part of the absorbent paper which is still dry and unused.

If you use a microplate washer or fully-automated ELISA, make sure that the machine is correctly adjusted, request settings from the manufacturer, if necessary. To avoid blocking the wash needles, only particle-free stool suspensions should be dispensed (see, PREPARATION OF SAMPLES). Also make sure that all liquid is aspirated during each wash step.

3. Second incubation

Use a pipette to fill 100 µl streptavidin poly-peroxidase conjugate, Conjugate 2, into the wells, then incubate for 15 minutes at room temperature (20 – 25 °C).

4. Washing

Wash as described in step 2.

5. Third incubation

Fill all wells with 100 µl substrate. Then, incubate the plate for 15 minutes in the dark at room temperature (20 - 25 °C). Next, fill all wells with 50 µl stop reagent to stop the reaction. After blending carefully by tapping lightly on the side of the plate, measure the extinction at 450 nm and at 620 nm reference wavelength.

Note: High-positive samples may cause black-colored precipitates of the substrate.

Quality Control

For quality control purposes, positive and negative controls must be used each time the test is carried out, to ensure that the reagents are stable and that the test is conducted correctly. The test has been performed correctly if the extinction rate (O.D.) for the negative control is less than 0.2 at 450 nm (less than 0.160 at 450/620 nm) and the measured value for the positive control is greater than 0.8 at 450 nm or at 450/620 nm. A value greater than 0.2 (0.160) for the negative control may indicate that washing was insufficient. Deviation from the required values, just like a turbid or blue coloring of the colorless substrate before it is filled into the wells, may indicate that the reagents have expired. If the stipulated values are not met, the following points must be checked before repeating the test:

Expiry date of the reagents

Functionality of the equipment (e.g. calibration)

Correct test procedure

Visual inspection of the kit components for contamination or leaks - a substrate solution which has turned blue must not be used.

Calculation

Calculating the cut-off:

To establish the cut-off, 0.15 extinction units are added to the measured extinction for the negative control.

Cut-off = extinction for the negative control + 0.15

Test results:

Sample assessment is positive if the extinction rate is greater than 10 % higher than the calculated cut-off value.

Sample assessment is borderline if the extinction rate ranges from 10 % less to 10 % greater than the cut-off value. If a repeat examination with a fresh stool sample again falls within the gray zone, the sample assessment is negative.

Samples with extinctions greater than 10 % below the calculated cut-off must be considered negative.

Precision

To determine intra-assay reproducibility, 40 replicates of these references were assayed, representing the complete measurement range from negative to high-positive. The mean values and the coefficients of variation (CV) were determined for three lots of the kits. For the interassay reproducibility, references from 10 different working days were assayed in duplicates, with two runs per day. The measurements were determined by three technicians for three lots of the kits. The inter-lot reproducibility was determined for all

three lots of the kits.

Reference		Intra-assay			Inter-assay			Inter-lot
		Kit Lot 1	Kit Lot 2	Kit Lot 3	Kit Lot 1	Kit Lot 2	Kit Lot 3	Kit Lot 1-3
1	MV [OD 450/620]	1.901	1.402	1.465	1.581	1.707	1.507	1.598
	CV (%)	6.1 %	8.4 %	5.9 %	17.0 %	16.7 %	18.7 %	18.2 %
2	MV [OD 450/620]	1.375	1.095	1.209	1.155	1.253	1.124	1.177
	CV (%)	5.8 %	7.5 %	5.7 %	13.7 %	14.9 %	16.3 %	15.7 %
3	MV [OD 450/620]	1.091	0.976	0.810	0.933	1.010	0.869	0.937
	CV (%)	5.3 %	10.6 %	6.1 %	16.2 %	15.2 %	14.0 %	16.7 %
4	MV [OD 450/620]	0.606	0.532	0.512	0.507	0.556	0.461	0.508
	CV (%)	5.5 %	6.4 %	7.9 %	19.5 %	14.3 %	17.2 %	19.1 %
5	MV [OD 450/620]	0.350	0.325	0.204	0.306	0.330	0.276	0.304
	CV (%)	6.6 %	12.2 %	16.9 %	19.8 %	17.5 %	21.2 %	20.9 %
6	MV [OD 450/620]	-0.001	-0.002	0.000	0.012	0.001	0.005	0.006
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Sensitivity

To determine the analytic sensitivity of the Entamoeba ELISA, a linear dilution series from a sample with a known quantity of Entamoeba cysts was produced and then measured in triplicates. The limit of detection (LoD) is the last concentration to be evaluated as positive in all replicates.

	E. histolytica		*E. dispar*	
	MV [OD 450/620]	Cysts / Reaction	MV [OD 450/620]	Cysts / Reaction
LoD	0.173	17	0.200	595

Specificity

A variety of pathogenic microorganisms from the intestinal tract were examined with the Entamoeba ELISA and except for Campylobacter coli, they showed no cross reactivity. These studies were conducted with undiluted bacteria or virus suspensions shown to have concentrations of 10^6 to 10^9 organisms per ml.

Interferences

The following list of substances showed no effects on the test results when they were blended into the supernatants of Entamoeba positive and Entamoeba negative stool samples in the described concentrations:

Mucin	5.0 % w/w	Diclofenac	0.1 % v/w
Human blood	5.0 % v/w	Cyclamate	1.3 % v/w
Pepto-Bismol	6.6 % v/w	Stearic acid / Palmitic acid	40 % w/w (1:1)
Loperamide	0.02 % w/w	Metronidazole	0.5 % solution

A possible dose effect relationship was investigated for barium sulfate (18.5 % w/w). This investigation of a serial dilution with barium sulfate, however, did not show a direct relationship between the concentration and the OD values. The only exception is the highest concentration that was tested, which is even higher than the "worst case" concentration that was tested in the first analysis (three times the daily dose). Interference due to barium sulfate can therefore be considered improbable.

Precautions

1. This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories must be followed. Always adhere strictly to the user instructions for this test.
2. Samples or reagents must not be pipetted by mouth and contact with injured skin or mucous membranes must be prevented.
3. Wear personal safety gear (suitable gloves, laboratory coat, safety glasses) when handling the samples, and wash hands after finishing the test.
4. Do not smoke, eat, or drink in areas where samples are being processed.
5. The kit includes a positive control that contains inactivated Entamoeba antigen. It must be treated as potentially infectious material and handled in accordance with the national safety regulations, just like the samples.
6. The wash buffer contains 0.1 % thimerosal as preservative. This substance must not come into contact with skin or mucous membranes.
7. Ensure the proper and responsible disposal of all reagents and materials after their use. For disposal, please adhere to national regulations!