Version 10-08/24

User's Manual

Human Echinococcus IgG ELISA Kit

REF **DEIA1777-2**

Σ **96T**

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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 Echinococcus granulosus ELISA kit is intended for the qualitative detection of IgG antibodies against Echinococcus granulosus (and Echinococcus multilocularis) in human serum.

General Description

Cystic echinocccosis, also known as hydatid disease, is caused by the larval stage of Echinococcus granulosus, a tapeworm found in dogs. Sheep, cattle, goats, and pigs as intermediate hosts harbor hydatid cysts mainly in liver and lungs. Humans can be infected by accidentally ingesting tapeworm eggs upon contaminated soil, food or water. The parasite larvae subsequently develop into hydatid cysts mainly in the liver and lungs as well. The main symptoms appear when hydatid cysts grow large enough to cause organ dysfunction plus discomfort, pain, nausea, and vomiting. Diagnosis is based on imaging techniques, such as CT scans, ultrasonography, and MRIs to detect the presence of well-delineated cyst(s) in a person with a history of exposure and a positive result by serological testing.

Principles of Testing

The kit provides all the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on breakable microtitration wells sensitized with Echinococcus granulosus hydatid fluid antigen. Specific antibodies in the sample will bind to these antigens and washing will remove unspecific antibodies. The presence of parasite specific antibodies is detected with a Protein A - alkaline phosphatase conjugate. A second washing step will remove unbound conjugate. Revealing bound antibodies is made by the addition of pNPP substrate which turns yellow in the presence of alkaline phosphatase. Color intensity is proportional to the amount of Echinococcusgranulosus specific antibodies in the sample. Potassium phosphate is added to stop the reaction. Absorbance at 405 nm is read using an ELISA microplate reader.

The test can be performed with automatic systems, but this must be validated by the user.

Reagents And Materials Provided

1. **WELL** Breakable ELISA strips sensitized with Echinococcus granulosus hydatid fluid antigen 96 wells
2. **DILB** Dilution buffer (10 x) concentrate, coloured purple 50 ml
3. **WASH** Washing solution (10 x) concentrate 50 ml
4. **ENZB** Enzyme buffer 50 ml
5. **STOP** Stopping solution (0.5M K_3PO_4) 25 ml
6. **CONTROL -** Negative control serum (20 x), green cap 200 µl
7. **CONTROL -/+** Weak positive control serum (cut off, 20 x), yellow cap 200 µl
8. **CONTROL +** Positive control serum (20 x), red cap 200 µl
9. **CONJ** Protein A - alkaline phosphatase conjugate (50 x), purple cap 300 µl
10. **SUBS** Phosphatase substrate (para-nitrophenylphosphate) 20 tablets

11. Multipipette reservoir, 25 ml 1 piece
12. Frame for ELISA 8-well holder 1 piece

Materials Required But Not Supplied

1. Pipettes (ml and µl).
2. Flasks.
3. Dilution tubes.
4. Adhesive tape to cover wells during incubations.
5. Distilled water.
6. Incubator set at 37°C.
7. ELISA reader set at 405 nm.
8. Manual or automatic equipment for rinsing wells.
9. Vortex mixer.
10. Timer.

Storage

Store the kit at 2° to 8°C (transport at ambient temperature), avoid long term exposure of the components to direct light. The expiry date and the lot number of the kit are printed on the side of the box. After initial opening, all reagents are stable until the expiry date when stored at 2-8°C.

Specimen Collection And Preparation

Use human serum. Serum should be stored at 2-8°C if analysed within a few days, otherwise store at –20°C or lower. Avoid repeated freezing and thawing.

Vortex samples and dilute 1/201 in dilution buffer solution (for instance 5 µl sample in 1.0 ml).

Reagent Preparation

1. Bring all reagents to room temperature and mix before use.
2. **ELISA wells**: open side of aluminum bag and remove number of wells needed (one for blank, three for controls plus the number of samples). Place sensitized wells in 8-well holder(s). If needed, complete the empty positions in the holder with used wells. Insert holder(s) in the frame in the correct orientation. Reseal open package with desiccant pad.
3. **Dilution buffer**: dilute dilution buffer (10 x) concentrate, 1/10 in distilled water. This is used for the dilution of controls, samples and conjugate. The diluted buffer is stable for 2 months at 2-8°C.
4. **Washing solution**: dilute washing solution (10 x) concentrate, 1/10 in distilled water. You may also use your own washing solution. Avoid buffers containing phosphate which could inhibit the enzymatic activity of the alkaline phosphatase. The diluted washing solution is stable for 2 months at 2-8°C.
5. **Control sera**: dilute 10 µl control sera in 190 µl dilution buffer solution (final dilution 1/20). The diluted control sera are stable for 2 months at 2-8°C.

6. **Conjugate**: dilute conjugate in dilution buffer solution (final dilution 1/50). Dilute conjugate on the day of the assay. Do not store diluted conjugate.
7. **Substrate solution**: dissolve tablet(s) of phosphatase substrate in undiluted enzyme buffer (1 tablet in 2.5 ml buffer). Vortex until complete dissolution of the tablet(s). Dilute substrate on the day of the assay and protect the tube from direct light. Tablets and substrate solutions should be colourless or should have only a slight yellow tinge. If a tablet or a substrate solution turns yellow, it may have been partially hydrolysed and should be discarded. Do not store the substrate solution.
8. **Stopping solution**: use reagent undiluted.

Assay Procedure

When running the assay, avoid the formation of bubbles in the wells.

Step 1: Blocking:

Fill completely wells with dilution buffer solution.

Incubate for 5 to 15 minutes at ambient temperature (blocking).

Remove dilution buffer by aspiration or by shaking the wells over the sink.

Step 2: Incubation with samples:

Fill the first well of the first strip with 100 µl dilution buffer only (no-serum blank).

Fill the subsequent three wells with respectively 100 µl diluted negative, weak positive (cut off) and positive control serum. For assays of more than 25 samples, we recommend to fill the three last wells with control sera as a duplicate.

Fill remaining wells with the diluted samples (100 µl each).

Cover wells with adhesive tape and incubate for 30 minutes at 37°C.

Remove sera and wash 4 x with ~ 250 µl washing solution.

Step 3: Incubation with conjugate:

Distribute 100 µl diluted conjugate in each well (including no-serum blank).

Cover wells with adhesive tape and incubate for 30 minutes at 37°C.

Remove conjugate and wash 4 x with ~ 250 µl washing solution.

Step 4: Incubation with substrate:

Distribute 100 µl substrate solution per well.

Cover wells with adhesive tape and incubate for 30 minutes at 37°C.

Stop the reaction by the addition of 100 µl stopping solution to each well.

Step 5: Measurement of absorbances:

If needed, wipe the bottom of the wells and eliminate bubbles. Measure absorbances at 405 nm within 1 hour after the addition of stopping solution.

Interpretation Of Results

Subtract the value of the no-serum blank from all measured values. When applicable calculate the mean absorbance values of duplicated serum controls. The test is valid if the following criteria are met:

- absorbance (A) of positive control > 1.200
- A of negative control < 10 % of A of positive control
- A of blank against air < 0.350

The antibody concentration of the weak positive (cut off) serum 9350-07 has been set to discriminate optimally between sera of clinically documented cases of echinococcosis and healthy human sera. The cut off index of a sample is defined, after subtraction of the no-serum blank, as:

Index = Absorbance sample / Absorbance cut off serum

The result is negative when the index of the analyzed sample is lower than 1.0. In this case, the antibody concentration against Echinococcus granulosus antigen is clinically non-significant. The result is positive when the index of the analyzed sample is higher than 1.0. In this case, the IgG antibody concentration against Echinococcus granulosus antigen is considered as clinically significant. It indicates that the patient has had a contact with the parasite. A grey zone could be defined by each laboratory according to its patients population. In case of borderline or doubtful results, we recommend repeating the test again 2-4 weeks later with a fresh sample.

Performance Characteristics

A sensitivity of 96% was found with 24 sera of patients suffering from cystic echinococcosis. A sensitivity of 89% was found with 19 sera of patients suffering from alveolar echinococcosis. Positive and doubtful results should be retested with the Echinococcus multilocularis-specific Em2-Em18 to identify the infecting Echinococcus species. A specificity of 97% was found with 119 sera of blood donnors (Swiss).

Precision

Repeatability were assessed by testing 2 human serum samples in 24 wells on 1 assay.

Reproducibility were assessed by testing the 2 human serum samples on 10 differents assays.

	Repeatability		Reproducibility	
	Sample 1	Sample 2	Sample 1	Sample 2
Average (absorbance)	0.586	1.416	0.684	1.654
Standard deviation (absorbance)	0.027	0.064	0.024	0.079
Variation coefficient (%)	4.6	4.5	3.5	4.8

Precautions

Toxic compounds are found in following:

1. Dilution buffer (10 x): Sodium azide (NaN_3) 0.1 % Merthiolate 0.02 %
2. Washing solution (10 x): Sodium azide (NaN_3) 0.05 %
3. Enzyme buffer: Sodium azide (NaN_3) 0.01 %
4, Control sera (20 x): Sodium azide (NaN_3) 0.1 % Merthiolate 0.02 %
5. Conjugate (50 x): Sodium azide (NaN_3) 0.1 %

6. At the used concentrations, sodium azide and merthiolate do not have any toxicological risk on contact with skin and mucous membranes.
7. The stopping solution (0.5 M K_3PO_4) is irritant.
8. The negative, weak positive, and positive control sera are from rabbits.
9. Treat all reagents and samples as potentially infectious material.
10. Do not interchange reagents of different lots or ELISA kits.
11. Do not use reagents from other manufacturers with reagents of this kit.
12. Do not use reagents after their expiry date.
13. Close reagent vials tightly immediately after use and do not interchange screw caps to avoid contamination.
14. Use separate and clean pipettes tips for each sample.
15. Do not re-use microwells.

Limitations

Internal evaluation showed that hemorragic, lipemic or icteric sera do not interfere with the results of the test.

A specificity of 82 % was found with 74 sera of patients with other parasitic infections. Cross-reactivity mainly occur in patients with fascioliasis, filariasis, leishmaniasis and cysticercosis.

In immunocompromised patients and newborns, serological data are of limited value.