ECHINOCOCCUS GRANULOSUS

Enzyme immunoassay for the diagnosis of human echinococcoses

96 assays on individual wells for in vitro use

CE

Instructions for use for article N° **9350**
EC reg. N°: H-CH/CA01/IVD/01757

Intended use:

Serological diagnosis (IgG) of human cystic hydatid disease (caused by ***Echinococcus granulosus***). This assay can also be used for the serological diagnosis of alveolar hydatid disease (caused by ***Echinococcus multilocularis***). Positive and doubtful cases should be retested with the *Echinococcus multilocularis*-specific Em2-Em18 (Bordier Affinity Products, article N° 9300) to identify the infecting *Echinococcus* species.

Principle and presentation:

The kit provides the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on microtitration wells sensitized with ***Echinococcus granulosus*** hydatid fluid antigen. The presence of parasite specific serum antibodies is detected with a Protein A - alkaline phosphatase conjugate. Sensitized wells are provided as breakable strips for the economical assay of small series of samples.

Material contained in the kit (96 assays):

WELL	9350-01	Breakable ELISA strips sensitized with ***Echinococcus granulosus*** antigen (hydatid fluid)	96	wells
DILB	9350-02	Dilution buffer (10 x) concentrate	50	ml
WASH	9350-03	Washing solution (10 x) concentrate	50	ml
ENZB	9350-04	Enzyme buffer	50	ml
STOP	9350-05	Stopping solution (K_3PO_4)	25	ml
CONTROL -	9350-06	Negative control serum	200	µl
CONTROL -/+	9350-07	Weak positive serum (cut off)	200	µl
CONTROL +	9350-08	Positive control serum	200	µl
CONJ	9350-09	Protein A - alkaline phosphatase conjugate	300	µl
SUBS	9350-10	Phosphatase substrate	20	tablets
		Multipipette reservoir, 25 ml	1	piece
		Frame for ELISA 8-well holder	1	piece

Shelf life and storage:
Store kit at 2° to 8° C (transport at ambient temperature). The expiry date and the lot number of the kit are printed on the side of the box.

Equipment needed but not provided with the kit:

Pipettes (ml and µl). Flasks. Tubes for the dilution of sera. Adhesive tape to cover wells during incubations. Distilled water. Incubator set at 37° C. ELISA reader set at 405 nm.

Preparation of reagents before use:

ELISA wells: open side of aluminum bag 9350-01 and remove number of wells needed. 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.

Dilution buffer: dilute dilution buffer (10 x) concentrate 9350-02, 1/10 in distilled water.

Washing solution: dilute washing solution (10 x) concentrate 9350-03, 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.

Negative, weak positive (cut off) and positive **control sera**: dilute 10 µl control sera 9350-06 to -08 in 190 µl dilution buffer solution (final dilution 1/20).

Sera to be tested: dilute 10 µl serum in 2.0 ml dilution buffer solution (final dilution 1/201).

Protein A - alkaline phosphatase **conjugate**: dilute conjugate 9350-09, in dilution buffer solution (final dilution 1/51).

Substrate solution: prewarm enzyme buffer 9350-04 at ambient temperature. Before the addition of substrate to the ELISA wells, dissolve tablet(s) of phosphatase substrate 9350-10 in undiluted buffer 9350-04 (1 tablet in 2.5 ml buffer). Vortex until complete dissolution of the tablet(s).

Stopping solution: use reagent 9350-05 undiluted.

Warnings and precautions: Solutions 9350-02, 9350-03, 9350-04 and 9350-09 contain respectively 0.1%, 0.05%, 0.01% and 0.1% of sodium azide (N_aN_3). Solution 9350-02 contain 0.02% of merthiolate. These substances are toxic. The stopping solution 9350-05 (0.5 M K_3PO_4) is irritant.

The negative, weak positive, and positive control sera (9350-06 to -08) are from rabbits.

Volumes to be prepared:

			Total number of wells to be used			
			3-4	**5-6**	**7-8**	**9-10**
Dilution buffer (10 x)	9350-02 + H_2O	ml + ml	1 + 9	2 + 18	3 + 27	4 + 36
Washing solution (10 x)	9350-03 + H_2O	ml + ml	1 + 9	2 + 18	3 + 27	4 + 36
Conjugate	9350-09 + dilution buffer	µl + µl	10 + 500	15 + 750	20 + 1000	25 + 1250
Control sera	9350-06 to -08 + dilution buffer	µl + µl	10 + 190	10 + 190	10 + 190	10 + 190
Sera to be tested	Serum + dilution buffer	µl + µl	10 + 2000	10 + 2000	10 + 2000	10 + 2000
Substrate solution	9350-10 + 9350-04	tabl. + ml	1 + 2.5	1 + 2.5	1 + 2.5	1 + 2.5

Procedure:

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 strips over the sink.

Step 2: Incubation with serum samples:

Fill the first well of the first strip with 100 µl dilution buffer only (no-serum blank). Fill the subsequent three wells with 100 µl diluted negative, weak positive (cut off) and positive control sera respectively (100 µl each). Fill remaining wells with the diluted sera to be tested (100 µl each). Cover wells with adhesive tape and incubate for 30 minutes at 37° C. Remove sera and wash 4 x with washing solution.

Step 3: Incubation with conjugate:

Distribute 100 µl diluted protein A - alkaline phosphatase conjugate in each well. Cover wells with adhesive tape and incubate for 30 minutes at 37° C. Remove conjugate and wash 4 x with 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:

Wipe bottom of wells, eliminate bubbles and measure absorbances at 405 nm.

Interpretation:

Subtract value of the no-serum blank from all measured values. The test is valid if the following criteria are met: absorbance (A) of positive control > 1.200, A of negative control < 12 % 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 cystic hydatid disease and healthy human sera.

The result is **negative** when the absorbance of the analysed sample is lower than the absorbance of the weak positive serum 9350-07. In this case, the IgG antibody concentration against the ***Echinococcus granulosus*** antigen is clinically non-significant.

The result is **positive** when the absorbance of the analysed sample is higher than the absorbance of the weak positive serum 9350-07. In this case, the IgG antibody concentration against the ***Echinococcus granulosus*** antigen is considered as clinically significant.

Sensitivity and specificity of the assay:

A diagnostic sensitivity of 96 % was found on a group of 24 patients with cystic hydatid disease (*Echinococcus granulosus*). Most patients (90 %) with alveolar hydatid disease (*Echinococcus multilocularis*) were also found positive with this assay.

The specificity of the assay with sera from patients with other parasitoses were tested. Results were negative with 82% of patients with helminthiases (n=51) and 83% with protozooses (n=23). 150 sera of blood donors (Swiss) were negative at 97 %. Internal evaluation showed that hemorragic, lipemic or icteric sera do not interfere with the results of the test.

Within the usual range of samples analyzed in a European laboratory, the predictive value of a negative result is close to 100 %. In contrast, a positive result has to be confirmed in all cases with more specific assays (Em2-Em18 and search for anti-8 Kd antibodies by immunoblot).

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

References:

Gottstein, B. (1992) Molecular and Immunological diagnosis of Echinococcosis. Clin. Microbiol. Rev. **5 :** 248-261.
Gottstein, B., Jacquier, P., Bresson-Hadni, S. and Eckert, J. (1993) Improved primary immunodiagnosis of Alveolar Echinococcosis in humans by an enzyme-linked immunosorbent assay using the Em2 ^{plus} antigen. J. Clin. Microbiol. **31:** 373-376.
Poretti, D., Felleisen, E., Grimm, F., Pfister, M., Teuscher, F., Zuercher, C., Reichen, J. and Gottstein, B. (1999) Differential immunodiagnosis between cystic hydatid disease and other cross-reactive pathologies. Am. J. Trop. Med. Hyg. **60:** 193-198.

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