ECHINOCOCCUS MULTILOCULARIS

Enzyme immunoassay for the diagnosis of human alveolar echinococcosis

96 assays on individual wells for in vitro use

CE

Instructions for use for article N° **9300**
EC reg. N°: H-CH/CA01/IVD/01757

Intended use:

Serological diagnosis (IgG) of human alveolar echinococcosis (alveolar hydatid disease). Sero-epidemiological surveys and examination of persons at risk, following exposition to infection. Post-operative control.

Principle and presentation:

The kit provides the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on microtitration wells sensitized with ***Echinococcus multilocularis*** Em2-Em18 antigens. 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.

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Material contained in the kit (96 assays):

WELL	9300-01	Breakable ELISA strips sensitized with ***Echinococcus multilocularis*** Em2-Em18 antigens	96	wells
DILB	9300-02	Dilution buffer (10 x) concentrate	50	ml
WASH	9300-03	Washing solution (10 x) concentrate	50	ml
ENZB	9300-04	Enzyme buffer	50	ml
STOP	9300-05	Stopping solution (K_3PO_4)	25	ml
CONTROL -	9300-06	Negative control serum	200	µl
CONTROL -/+	9300-07	Weak positive serum (cut off)	200	µl
CONTROL +	9300-08	Positive control serum	200	µl
CONJ	9300-09	Protein A - alkaline phosphatase conjugate	300	µl
SUBS	9300-10	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 9300-01 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 9300-02, 1/10 in distilled water.

Washing solution: dilute washing solution (10 x) concentrate 9300-03 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 9300-06 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 9300-09, 1/51 in dilution buffer solution (final dilution 1/51).

Substrate solution: prewarm enzyme buffer 9300-04 at ambient temperature. Before the addition of substrate to the ELISA wells, dissolve tablet(s) of phosphatase substrate tablets 9300-10 in undiluted buffer 9300-04 (1 tablet in 2.5 ml buffer). Vortex until complete dissolution of the tablet(s).

Stopping solution: use reagent 9300-05 undiluted.

Warnings and precautions: Solutions 9300-02, 9300-03, 9300-04 and 9300-09 contain respectively 0.1%, 0.05%, 0.01% and 0.1% of sodium azide (N_aN_3). Solution 9300-02 contain 0.02% of merthiolate. These substances are toxic. The stopping solution 9300-05 (0.5 M K_3PO_4) is irritant.

The negative, weak positive, and positive control sera (9300-06 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)	9300-02 + H_2O	ml + ml	1 + 9	2 + 18	3 + 27	4 + 36
Washing solution (10 x)	9300-03 + H_2O	ml + ml	1 + 9	2 + 18	3 + 27	4 + 36
Conjugate	9300-09 + dilution buffer	µl + µl	10 + 500	15 + 750	20 + 1000	25 + 1250
Control sera	9300-06 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	9300-10 + 9300-04	tabl. + ml	1 + 2.5	1 + 2.5	1 + 2.5	1 + 2.5

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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.

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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 < 15 % of A of positive control, A of blank against air < 0.350.

The antibody concentration of the weak positive (cut off) serum 9300-07 has been set to discriminate optimally between sera of clinically documented cases of alveolar echinococcosis and healthy human sera.

The result is **negative** when the absorbance of the analyzed sample is lower than the absorbance of the weak positive serum 9300-07. In this case, the IgG antibody concentration against ***Echinococcus multilocularis*** Em2-Em18 antigens is clinically non-significant.

The result is **positive** when the absorbance of the analyzed sample is higher than the absorbance of the weak positive serum 9300-07. In this case, the IgG antibody concentration against ***Echinococcus multilocularis*** Em2-Em18 antigens is considered as clinically significant.

Sensibility and specificity of the assay:

A diagnostic sensitivity of 83 % was found on a group of 151 patients with alveolar echinococcosis (*Echinococcus multilocularis*). Approximately 84 % (n = 63) of cystic echinococcoses (*E. granulosus*) are negative with this test.

The specificity of the assay with sera from patients with other parasitoses was 93 % (n=46). 267 sera of blood donors (Swiss) were negative at 98 %. Internal evaluation showed that hemorragic, lipemic or icteric sera do not interfere with the results of the test.

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)	0.647	1.518	0.580	1.407
Standard deviation (absorbance)	0.033	0.058	0.026	0.064
Variation coefficient (%)	5.1	3.8	4.5	4.6

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References:

Müller, N., Gottstein, B., Vogel, M., Flury, K. and Seebeck, T. (1989) Application of a recombinant *Echinococcus multilocularis* antigen in an ELISA for immunodiagnosis of human alveolar echinococcosis. Mol. Biochem. Parasitol. **36 :** 151-160.
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Eckert, J., Conraths, F. and Tackmann, K. (2000) Echinococcosis: an emerging or re-emerging zoonosis? Int. J. Parasitol. **30 :** 1283-1294.
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Knapp, J., Sako, Y., Grenouillet, F. Bresson-Hadni, S. Richou, C., Gbaguidi-Haore, H., Ito, A., Millon, L. (2014) Comparison of the serological tests ICT and ELISA for the diagnosis of alveolar echinococcosis in France. Parasite. **21.**

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