ECHINOCOCCUS MULTILOCULARIS recEm-18

Enzyme immunoassay for the serological follow-up of human alveolar echinococcosis

96 assays on individual wells for in vitro use

CE

Instructions for use for article N° **9310**
EC reg. N°: CH-201708-0010

Intended use:

Post-operative and/or post-therapeutic serological follow-up (IgG) of human alveolar echinococcosis. A significant decrease or even a negativation of specific anti-Em-18 serum antibodies indicates an inactivation of the parasite, especially when connected with a negativation in PET-imaging.

Principle and presentation:

The kit provides the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on microtitration wells sensitized with ***Echinococcus multilocularis*** Em-18 recombinant 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	9310-01	Breakable ELISA strips sensitized with ***Echinococcus multilocularis*** Em-18 antigen	96	wells
DILB	9310-02	Dilution buffer (10 x) concentrate	50	ml
WASH	9310-03	Washing solution (10 x) concentrate	50	ml
ENZB	9310-04	Enzyme buffer	50	ml
STOP	9310-05	Stopping solution (K_3PO_4)	25	ml
CONTROL -	9310-06	Negative control serum	200	µl
CONTROL -/+	9310-07	Weak positive serum (cut-off)	200	µl
CONTROL +	9310-08	Positive control serum	200	µl
CONJ	9310-09	Protein A - alkaline phosphatase conjugate	300	µl
SUBS	9310-10	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 9310-01 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 9310-02, 1/10 in distilled water.

Washing solution: dilute washing solution (10 x) concentrate 9310-03 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 9310-06 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 9310-09, 1/51 in dilution buffer solution (final dilution 1/51).

Substrate solution: prewarm enzyme buffer 9310-04 at ambient temperature. Before the addition of substrate to the ELISA wells, dissolve tablet(s) of phosphatase substrate tablets 9310-10 in undiluted buffer 9310-04 (1 tablet in 2.5 ml buffer). Vortex until complete dissolution of the tablet(s).

Stopping solution: use reagent 9310-05 undiluted.

Warnings and precautions: Solutions 9310-02, 9310-03, 9310-04 and 9310-09 contain respectively 0.1%, 0.05%, 0.01% and 0.1% of sodium azide (N_aN_3). Solution 9310-02 contain 0.02% of merthiolate. These substances are toxic. The stopping solution 9310-05 (0.5 M K_3PO_4) is irritant.

The negative, weak positive, and positive control sera (9310-06 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)	9310-02 + H_2O	ml + ml	1 + 9	2 + 18	3 + 27	4 + 36
Washing solution (10 x)	9310-03 + H_2O	ml + ml	1 + 9	2 + 18	3 + 27	4 + 36
Conjugate	9310-09 + dilution buffer	µl + µl	10 + 500	15 + 750	20 + 1000	25 + 1250
Control sera	9310-06 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	9310-10 + 9310-04	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 < 5 % of A of positive control, A of blank against air < 0.350.

The antibody concentration of the weak positive (cut-off) serum 9310-07 has been set to discriminate optimally between sera of clinically documented cases of alveolar echinococcosis and healthy or under remission human sera.
The cut-off index of a sample is defined, after subtraction of the no-serum blank, as:

$$\text{Index} = \frac{\text{Absorbance sample}}{\text{Absorbance cut-off serum}}$$

The result is **negative** when the index of the analyzed sample is lower than **1.0**. In this case, the IgG antibody concentration against ***Echinococcus multilocularis*** Em-18 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 multilocularis*** Em-18 antigen is considered as clinically significant.
Decrease or negativation of anti-recEm18 serum antibody levels indicates a complete surgical resection of the parasite lesion or an inactivation of the parasite by drug treatment.

Sensibility and specificity of the assay:

Paired pre- and post-surgical serum samples of 12 patients with confirmed alveolar echinococcosis and having had a radical or non-radical surgery were studied. Pre-surgically, 9 patients (75%) had an index >1. Among these patients, 5 had negative post-surgical results. But in all 12 patients, post-surgical Em18 antibody levels dropped and were significantly lower than in pre-surgical samples.
Serum samples of 25 patients with confirmed alveolar echinococcosis without surgery but with stable disease under antiparasitic chemotherapy were studied. 18 (72%) of them had an index >1 (median index 6.3).
Serum samples of 7 patients with confirmed alveolar echinococcosis without surgery but with progressive disease under antiparasitic chemotherapy were studied. 6 (86%) of them had an index >1 (median index 13.8).

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)	0.186	1.260	0.157	1.123
Standard deviation (absorbance)	0.022	0.072	0.016	0.074
Variation coefficient (%)	11.8	5.7	10.0	6.5

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51304_01 9310 Eng 08.2016