FASCIOLA HEPATICA

Enzyme immunoassay for the diagnosis of human Fasciolosis

96 assays on individual wells for in vitro use

Instructions for use for article N° **9650**
EC reg. N°: CH-201504-0006

CE

Intended use:

Serological diagnosis (IgG) of human fasciolosis.

Principle and presentation:

The kit provides the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on microtitration wells sensitized with ***Fasciola hepatica*** 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	9650-01	Breakable ELISA strips sensitized with ***Fasciola hepatica*** recombinant antigen	96	wells
DILB	9650-02	Dilution buffer (10 x) concentrate	50	ml
WASH	9650-03	Washing solution (10 x) concentrate	50	ml
ENZB	9650-04	Enzyme buffer	50	ml
STOP	9650-05	Stopping solution (K_3PO_4)	25	ml
CONTROL -	9650-06	Negative control serum	200	µl
CONTROL -/+	9650-07	Weak positive serum (cut off)	200	µl
CONTROL +	9650-08	Positive control serum	200	µl
CONJ	9650-09	Protein A - alkaline phosphatase conjugate	300	µl
SUBS	9650-10	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 aluminium bag 9650-01 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 9650-02, 1/10 in distilled water.

Washing solution: dilute washing solution (10 x) concentrate 9650-03, 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 9650-06 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 9650-09 in dilution buffer solution (final dilution 1/51).

Substrate solution: prewarm enzyme buffer 9650-04 at ambient temperature. Before the addition of substrate to the ELISA wells, dissolve tablet(s) of phosphatase substrate 9650-10 in undiluted buffer 9650-04 (1 tablet in 2.5 ml buffer). Vortex until complete dissolution of the tablet(s).

Stopping solution: use reagent 9650-05 undiluted.

Warnings and precautions: Solutions 9650-02, 9650-03, 9650-04 and 9650-09 contain respectively 0.1%, 0.05%, 0.01% and 0.1% sodium azide (N_aN_3). Solution 9650-02 contain 0.02% merthiolate. These substances are toxic. The stopping solution, 9650-05 (0.5 M K_3PO_4) is irritant.

The negative, weak positive, and positive control sera (9650-06 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)	9650-02 + H_2O	ml + ml	1 + 9	2 + 18	3 + 27	4 + 36
Washing solution (10 x)	9650-03 + H_2O	ml + ml	1 + 9	2 + 18	3 + 27	4 + 36
Conjugate	9650-09 + dilution buffer	µl + µl	10 + 500	15 + 750	20 + 1000	25 + 1250
Control sera	9650-06 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	9650-10 + 9650-04	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 < 8% of A of positive control, A of blank against air < 0.350.

The antibody concentration of the weak positive (cut off) serum 9650-07 has been set to discriminate optimally between sera of clinically documented cases of fasciolosis 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 9650-07. In this case, the IgG antibody concentration against ***Fasciola hepatica*** recombinant antigen is clinically non-significant.

The result is **positive** when the absorbance of the analyzed sample is higher than the absorbance of the weak positive control 9650-07. In this case, the IgG antibody concentration against ***Fasciola hepatica*** recombinant antigen is considered as clinically significant. The interpretation of this result should take into consideration the cross-reactivities of other parasitic infections (undermentioned), the clinical symptoms and the endemic situation.

Sensitivity and specificity of the assay:

A **sensitivity** of **77%** was found with 13 sera from patients with fasciolosis.
A **specificity** of **99 %** was found with 99 sera of blood donors (Swiss).
A **specificity** of **98 %** was found with 100 sera from patients of an infectiology unit (Swiss).
The test of 30 patients with other parasitc infections showed a **specificity** of **97%**. Filariosis (0/12), hydatidosis (0/5), Bilharzia (0/1), and alveolar echinococcosis (1/12).

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 different assays.

	Repeatability		**Reproducibility**	
	Sample 1	**Sample 2**	**Sample 1**	**Sample 2**
Average (OD value)	0.459	1.491	0.469	1.547
Standard deviation (OD value)	0.023	0.089	0.028	0.069
Variation coefficient (%)	5.0	6.0	5.9	4.5

References:

Figueroa-Santiago, O., Delgado, B. and Espino, A.M. (2011) Fasciola hepatica saposin-like protein-2-based ELISA for the serodiagnosis of chronic human fascioliais. Diagnostic Microbiology and Infectious Disease 70, 355-361.

Gottstein B, Schneeberger M, Boubaker G, Merkle B, Huber C, et al. (2014) Comparative Assessment of ELISAs Using Recombinant Saposin-Like Protein 2 and recombinant Cathepsin L-1 from Fasciola hepatica for the Serodiagnosis of Human Fasciolosis. PLoS Negl Trop Dis 8(6): e2860. doi:10.1371/journal.pntd.0002860

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