ENTAMOEBA HISTOLYTICA

Enzyme immunoassay for the diagnosis of visceral amebiasis

96 assays on individual wells for in vitro use

CE

Instructions for use for article N° **9550**
EC reg. N°: CH-201202-0033

Intended use:

Serological diagnosis (IgG) of extra-intestinal amoebiasis by detection of specific antibodies in travelers retourning from endemic areas and developing symptoms like abdominal pain, fever and hepatomegaly. Post therapeutic controls.

Principle and presentation:

The kit provides the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on microtitration wells sensitized with ***Entamoeba histolytica*** soluble trophozoite 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.

Material contained in the kit (96 assays):

WELL	9550-01	Breakable ELISA strips sensitized with ***Entamoeba histolytica*** soluble antigens	96	wells
DILB	9550-02	Dilution buffer (10 x) concentrate	50	ml
WASH	9550-03	Washing solution (10 x) concentrate	50	ml
ENZB	9550-04	Enzyme buffer	50	ml
STOP	9550-05	Stopping solution (K_3PO_4)	25	ml
CONTROL -	9550-06	Negative control serum	200	µl
CONTROL -/+	9550-07	Weak positive serum (cut off)	200	µl
CONTROL +	9550-08	Positive control serum	200	µl
CONJ	9550-09	Protein A - alkaline phosphatase conjugate	300	µl
SUBS	9550-10	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 9550-01 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 9550-02, 1/10 in distilled water.

Washing solution: dilute washing solution (10 x) concentrate 9550-03, 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 9550-06 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 9550-09 in dilution buffer solution (final dilution 1/51).

Substrate solution: prewarm enzyme buffer 9550-04 at ambient temperature. Before the addition of substrate to the ELISA wells, dissolve tablet(s) of phosphatase substrate 9550-10 in undiluted buffer 9550-04 (1 tablet in 2.5 ml buffer). Vortex until complete dissolution of the tablet(s).

Stopping solution: use reagent 9550-05 undiluted.

Warnings and precautions: Solutions 9550-02, 9550-03, 9550-04 and 9550-09 contain respectively 0.1%, 0.05%, 0.01% and 0.1% of sodium azide (N_aN_3). Solution 9550-02 contain 0.02% of merthiolate. These substances are toxic. The stopping solution 9550-05 (0.5 M K_3PO_4) is irritant.

The negative, weak positive, and positive control sera (9550-06 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)	9550-02 + H_2O	ml + ml	1 + 9	2 + 18	3 + 27	4 + 36
Washing solution (10 x)	9550-03 + H_2O	ml + ml	1 + 9	2 + 18	3 + 27	4 + 36
Conjugate	9550-09 + dilution buffer	µl + µl	10 + 500	15 + 750	20 + 1000	25 + 1250
Control sera	9550-06 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	9550-10 + 9550-04	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 < 14% of A of positive control, A of blank against air < 0.350.

The antibody concentration of the weak positive (cut off) serum 9550-07 has been set to discriminate optimally between sera of clinically documented cases of amebiasis 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 9550-07. In this case, the IgG antibody concentration against ***Entamoeba histolytica*** soluble 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 9550-07. In this case, the IgG antibody concentration against ***Entamoeba histolytica*** soluble antigens is considered as clinically significant.

Sensitivity and specificity of the assay:

The sensitivity of the test was calculated at 100% with 52 sera from patients suffuring from visceral amoebiasis.
A specificity of 96% is observed with 99 sera from Swiss blood donors.
A specificity of 88,7% is observed with 71 sera from amebiasis suspected patients, but where this disease has been certainly ruled out.
A specificity of 80% is observed with 40 sera from patients suffering from other parasitic diseases. Most of the cross reactions are caused by leishmaniasis, malaria, filariasis and strongyloidiasis.
Internal evaluation showed that hemorragic, lipemic or icteric sera do not interfere with the results of the test.
In any case, it is necessary to integrate all the clinical, epidemiological, radiological and biological data before establishing the final diagnosis.

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 (OD value)	0.612	2.394	0.649	2.449
Standard deviation (OD value)	0.040	0.162	0.041	0.162
Variation coefficient (%)	6.5	6.8	6.3	6.6

References:

Nicholls, R.S., I Restrepo, M., Duque, S., Consuelo Lopez, M., Corredor, A. (1994) Standardization and evaluation of Elisa for the serodiagnostic of amoebic liver abscess. Mem Inst Oswaldo Cruz, Rio de Janeiro. 89: 53-58.

Visser, L.G., Verweij, J.J., Van Esbroeck, M., Edeling, W.M., Clerinx, J. Polderman A.M. (2006) Diagnostic methods for differentiation of E. histolytica and E. dispar in carriers : performance and clinical implications in a non-endemic setting. Int. journal of med. microbiol. 296 : 397-403.

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