

# SCHISTOSOMA MANSONI

## Enzyme immunoassay for the diagnosis of human Schistosomiasis

96 assays on individual wells for in vitro use

Instructions for use for article N° 9600  
EC reg. N°: H-CH/CA01/IVD/17983



### Intended use:

Serological diagnosis (IgG) of schistosomiasis, especially during the invasive phase when few or no eggs are excreted, by detection of specific antibodies in travelers returning from endemic areas, with frequent contact with fresh water. Screening of travel companions of confirmed cases. Control programs in endemic areas. 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 *Schistosoma mansoni* soluble 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):

<b>WELL</b>	9600-01	Breakable ELISA strips sensitized with <i>Schistosoma mansoni</i> soluble antigens	96	wells
<b>DILB</b>	9600-02	Dilution buffer (10 x) concentrate	50	ml
<b>WASH</b>	9600-03	Washing solution (10 x) concentrate	50	ml
<b>ENZB</b>	9600-04	Enzyme buffer	50	ml
<b>STOP</b>	9600-05	Stopping solution (K <sub>3</sub> PO <sub>4</sub> )	25	ml
<b>CONTROL</b> -	9600-06	Negative control serum	200	µl
<b>CONTROL</b> -/+	9600-07	Weak positive serum (cut off)	200	µl
<b>CONTROL</b> +	9600-08	Positive control serum	200	µl
<b>CONJ</b>	9600-09	Protein A - alkaline phosphatase conjugate	300	µl
<b>SUBS</b>	9600-10	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  $\mu$ l). 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 9600-01 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 9600-02, 1/10 in distilled water.

**Washing solution:** dilute washing solution (10 x) concentrate 9600-03, 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  $\mu$ l control sera 9600-06 to -08 in 190  $\mu$ l dilution buffer solution (final dilution 1/20).

**Sera to be tested:** dilute 10  $\mu$ l serum in 2.0 ml dilution buffer solution (final dilution 1/201).

Protein A - alkaline phosphatase **conjugate:** dilute conjugate 9600-09 in dilution buffer solution (final dilution 1/51).

**Substrate solution:** prewarm enzyme buffer 9600-04 at ambient temperature. Before the addition of substrate to the ELISA wells, dissolve tablet(s) of phosphatase substrate 9600-10 in undiluted buffer 9600-04 (1 tablet in 2.5 ml buffer). Vortex until complete dissolution of the tablet(s).

**Stopping solution:** use reagent 9600-05 undiluted.



**Warnings and precautions:** Solutions 9600-02, 9600-03, 9600-04 and 9600-09 contain respectively 0.1%, 0.05%, 0.01% and 0.1% of sodium azide ( $N_3Na$ ). Solution 9600-02 contain 0.02% of merthiolate. These substances are toxic. The stopping solution 9600-05 (0.5 M  $K_3PO_4$ ) is irritant.

The negative, weak positive, and positive control sera (9600-06 to -08) are from rabbits.

## Volumes to be prepared:

			Total number of wells to be used			
			3-4	5-6	7-8	9-10
<b>Dilution buffer (10 x)</b>	9600-02 + H <sub>2</sub> O	ml + ml	1 + 9	2 + 18	3 + 27	4 + 36
<b>Washing solution (10 x)</b>	9600-03 + H <sub>2</sub> O	ml + ml	1 + 9	2 + 18	3 + 27	4 + 36
<b>Conjugate</b>	9600-09 + dilution buffer	$\mu$ l + $\mu$ l	10 + 500	15 + 750	20 + 1000	25 + 1250
<b>Control sera</b>	9600-06 to -08 + dilution buffer	$\mu$ l + $\mu$ l	10 + 190	10 + 190	10 + 190	10 + 190
<b>Sera to be tested</b>	Serum + dilution buffer	$\mu$ l + $\mu$ l	10 + 2000	10 + 2000	10 + 2000	10 + 2000
<b>Substrate solution</b>	9600-10 + 9600-04	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 9600-07 has been set to discriminate optimally between sera of clinically documented cases of schistosomiasis 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 9600-07. In this case, the IgG antibody concentration against **Schistosoma mansoni** 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 control 9600-07. In this case, the IgG antibody concentration against **Schistosoma mansoni** soluble antigens 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:

The test detects also *Schistosoma haematobium* infected patients.

A **sensitivity** of **94%** was found with 80 sera from patients with parasitologically proven schistosomiasis (34/37 *Schistosoma mansoni*, 26/27 *Schistosoma haematobium* and 2/3 mixed infections) or positive specific serology on western-blot(13/13).

A **specificity** of **99 %** was found with 122 sera of blood donors (Swiss).

The test of 141 patients with other parasitic infections showed a **specificity** of **94%**. Amebiasis (0/8), cysticercosis (0/3), fascioliasis (0/5), filariasis (3/21), hydatidosis (1/27), leishmaniasis (3/11), strongyloidosis (0/13), trichinellosis (1/9), toxocarosis (0/5), toxoplasmosis (1/20) and malaria (0/19).

Internal evaluation showed that hemorrhagic, 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 different assays.

	Repeatability		Reproducibility	
	Sample 1	Sample 2	Sample 1	Sample 2
<b>Average (absorbance)</b>	0.412	1.249	0.407	1.246
<b>Standard deviation (absorbance)</b>	0.031	0.067	0.029	0.076
<b>Variation coefficient (%)</b>	7.6	5.3	7.1	6.1

## References:

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