Version 26-11/25

User's Manual

Schistosoma mansoni IgG ELISA KIT

REF **DEIA05563**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Schistosoma mansoni IgG-ELISA is intended for the qualitative determination of IgG class antibodies against Schistosoma mansoni in human serum or plasma (citrate)

Principles of Testing

The qualitative immunoenzymatic determination of IgG-class antibodies against Schistosoma mansoni is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microtiter strip wells are precoated with Schistosoma mansoni antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled Protein A conjugate is added. This conjugate binds to antigen-antibody complexes. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.

The intensity of this product is proportional to the amount of Schistosoma-specific IgG antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell plate reader.

Reagents And Materials Provided

1. Microtiterplate, Schistosoma mansoni Coated Wells (IgG): 12 breakapart 8-well snap-off strips coated with Schistosoma mansoni antigens; in resealable aluminium foil.

2. Sample Diluent: 1 bottle containing 100 ml of buffer for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap.

3. Stop Solution: 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.

4. Washing Solution (20× conc.): 1 bottle containing 50 ml of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.

5. Conjugate: 1 bottle containing 20 ml of peroxidase Protein A; coloured blue, ready to use; black cap.

6. TMB Substrate Solution: 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; yellow cap.

7. Schistosoma mansoni IgG Positive Control: 1 bottle containing 2 ml; coloured yellow; ready to use; red cap.

8. Schistosoma mansoni IgG Cut-off Control: 1 bottle containing 3 ml; coloured yellow; ready to use; green cap.

9. Schistosoma mansoni IgG Negative Control: 1 bottle containing 2 ml; coloured yellow; ready to use; blue cap.

Materials Required But Not Supplied

1. ELISA Microtiterplates reader, equipped for the measurement of absorbance at 450/620 nm

2. Incubator 37°C
3. Manual or automatic equipment for rinsing Microtiterplates
4. Pipettes to deliver volumes between 10 and 1000 uL
5. Vortex tube mixer
6. Distilled water
7. Disposable tubes

Storage

Store the kit at 2-8°C. The opened reagents are stable up to the expiry date stated on the label when stored at 2-8°C.

Specimen Collection And Preparation

Use human serum or plasma (citrate, heparin) samples with this assay, If the assay is performed within 5 days after sample collection, the samples should be kept at 2-8°C; otherwise, they should be aliquoted and stored deep-frozen (-70 - -20°C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing. Heat inactivaton of samples is not recommended.

1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with Sample Dilution. Dispense 10 uL sample and 1 mL Sample Dilution into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

Reagent Preparation

It is very important to bring all reagents and samples to room temperature (20-25°C) and mix them before starting the test run!

1. Microtiterplate

The break-apart snap-off strips are coated with Schistosoma mansoni antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2-8°C.

2. Washing Solution (20× conc.)

Dilute Washing Solution (20×) 1+19, e.g. 10 mL Washing Solution (20×)+ 190 mL distilled water.The diluted buffer (Washing Solution 1×) stable for 5 days at room temperature (20-25°C). In case crystals appear in the concentrate, warm up the solution to 37 C e.g. in a water bath. Mix well before dilution.

3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2-8°C, away from the light. TMB Substrate Solution should be colourless or could have a slight blue tinge. If TMB Substrate Solution turms into blue, it may have become contaminated and should be thrown away.

Assay Procedure

Please read the instructions for use carefully before performing the assay. Result reliability depends on strict

adherence to the instructions for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Solution from 300 µL to 350 µL to avoid washing effects. Pay attention to Precautions. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37±1°C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit
3. Incubate for 1hour ± 5 min at 37± 1°C.
4. When incubaton has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Solution 1×. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step! **Note: Washing is important! Insufficient washing results in poor precision and false results.**
5. Dispense 100 µL Protein A conjugate into all wells except for the Substrate Blank well A1.
6. Incubate for 30 min at room temperature(20-25°C). Do not expose to direct sunlight.
7. Repeat step 4
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. Incubate for exactly 15 min at room temperature (20-25°C) in the dark. A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of Stop Solution.

Adjust the ELISA Microtiterplate reader to zero using the Substrate Blank.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard/control and sample.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

Quality Control

In order for an assay run to be considered valid, these instructions for use have to be strictly followed and the following criteria must be met:

Substrate Blank:Absorbance value < 0.100

Negative Control: Absorbance value < 0.200 and < Cut-off

Cut_off Control:Absorbance value 0.150 -1.300

Positive Control: Absorbance value > Cut_off

If these criteria are not met, the test is not valid and must be repeated.

Calculation

1. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example:

Absorbance value Cut-off Control 0.44 +absorbance value Cut_offcontrol 0.42 = 0.86/2 = 0.43

Cut-off= 0.43

2. Results in Units [CDU]

Sample (mean) absorbance value × 10 / Cut-off = (CD Units = CDU]

Example: 1.591×10 / 0.43 = 37CDU

Interpretation Of Results

Cut-off: 10 CDU

Positive: >11 CDU

Equivocal: 9-11 CDU

Negative: <9 CDU

Precision

Intraassay	n	Mean (E)	CV(%)
1	24	0.604	3.88
2	24	1.710	3.14
3	24	1.593	2.66

Interassay	n	Mean (E)	CV(%)
1	12	30.42	5.97
2	12	37.98	10.34
3	12	2.50	10.71