Human Schistosoma mansoni IgG antibody ELISA Kit

Enzyme Immunoassay for the qualitative screening of IgG antibodies to Schistosoma mansoni in serum or plasma.

Catalog number: ARG80607

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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PRINCIPLE OF THE ASSAY

This assay employs the qualitative enzyme immunoassay technique. Schistosoma mansoni antigen has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Ab present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated protein A is added to each well and incubate. After washing away any unbound protein A-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Ab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C.
Control A (Negative Control)	2 ml (Ready-to-use)	4°C
Control B (Cut-off Control)	3 ml (Ready-to-use)	4°C
Control C (Positive Control)	2 ml (Ready-to-use)	4°C
HRP-Streptavidin conjugate	20 ml (Ready-to-use)	4°C
20X Wash buffer	50 ml	4°C
Sample Diluent Buffer	100 ml	4°C
TMB substrate	15 ml	4°C (Protect from light)
STOP solution	15 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer.
- **Patient sample**: Dilute patient sample 1:101 with Sample diluent buffer before assay, mix well. (e.g. 5 µl of serum + 500 µl of sample diluent buffer)

  Note: the controls are ready-to-use and need not further dilution.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Leave 1 well for substrate blank, 1 well for the negative control, 2 wells for the Cut-off control and 1 well for the positive control.
3. Add 100 µl of diluted samples and controls into respective wells.
4. Incubate for 60 minutes at 37°C.
5. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add 100 µl 1X HRP-Streptavidin conjugate solution into each well (expect the substrate blank). Incubate for 30 minutes at RT.
7. Wash as according to step 5.
8. Add 100 µl of TMB Reagent to each well. Incubate for 15 minutes at room temperature.
9. Add 100 µl of Stop Solution to each well.
10. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

Adjust the ELISA microplate or microstrip reader to zero using the substrate blank in well A1. If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

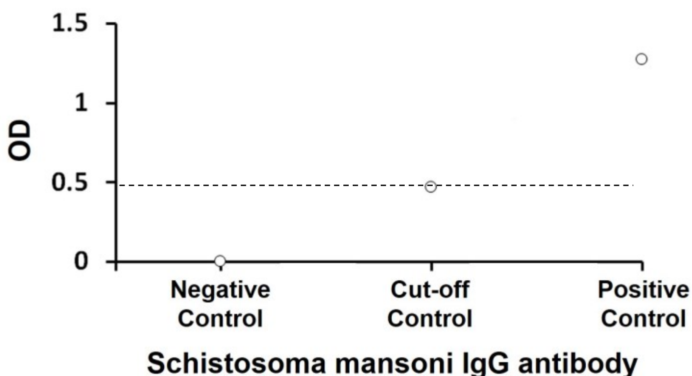
Measure the absorbance of all wells at 450 nm and record the absorbance values for each control and patient sample in the distribution and identification plan.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



INTERPRETATION OF RESULTS

CO = Mean of OD of Cut-off value

POSITIVE: Patient (mean) absorbance values more than 10 % above CO

GREY ZONE: Patient (mean) absorbance values from 10 % above or below CO repeat test 2 - 4 weeks later with new patient samples

Results in the second test again in the grey zone ⇒ NEGATIVE

NEGATIVE: Patient (mean) absorbance values more than 10 % below CO

$$\frac{Patient\ (mean) absorbance\ value \times 10}{CO} = Unit;\ U$$

Cut-off value: 10 U

Grey zone: 9 - 11 U

Negative: < 9 U

Positive: > 11 U

QUALITY ASSURANCE

Interferences

Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 5.7% and inter-assay precision was 5.1%.