Human Schistosoma antibody IgG ELISA Kit

Catalog number: NR-R10017 (96 wells)

The kit is intended for qualitatively detection of Schistosoma antibody IgG in serum, plasma and other suitable sample solution

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES
Background

Schistosomiasis, also known as bilharzia, is a disease caused by parasitic worms. The parasites that cause schistosomiasis live in certain types of freshwater snails. The infectious form of the parasite, known as cercariae, emerge from the snail, hence contaminating water. Most human infections are caused by Schistosoma mansoni, S. haematobium, or S. japonicum. Infection occurs when skin comes in contact with contaminated freshwater in which certain types of snails that carry the parasite are living. Schistosoma parasites can penetrate the skin. Over several weeks, the parasites migrate through host tissue and develop into adult worms inside the blood vessels of the body. Most people have no symptoms when they are first infected. However, within days after becoming infected, they may develop a rash or itchy skin. Within 1-2 months of infection, symptoms may develop including fever, chills, cough, and muscle aches.

Intended use

The kit is used to perform a qualitative test of Schistosoma antibody IgG in serum, plasma and other suitable sample solution.

<table>
<thead>
<tr>
<th>Assay time</th>
<th>1.5 hours</th>
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<tbody>
<tr>
<td>Validity</td>
<td>12 months</td>
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<tr>
<td>Store at</td>
<td>2-8 °C</td>
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Assay principle

This Schistosoma antibody IgG ELISA Kit was based on standard sandwich enzyme-linked immunosorbent assay technology. Purified Schistosoma antigen was precoated onto 96-well plates. Human Schistosoma antibodies, if presented in test samples, would bind to Schistosoma antigen precoated on the plate. Then HRP conjugated rabbit anti-human IgG antibody was added and unbound conjugates were washed away with wash buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the Schistosoma IgG amount of sample captured in plate.

Materials supplied

| 1 | Microelisa Stripplate |
| 2 | Positive control |
| 3 | Negative Control |
| 4 | 20 X Wash Solution |
| 5 | Assay diluent |
| 6 | HRP conjugate |
| 7 | TMB substrate |
| 8 | Stop Solution |
| 9 | Package insert |
Materials required but not supplied

- Standard plate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Distilled water.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Absorbent paper.
- Materials used for sample preparation.

Sample collection and storage

- **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before a centrifugation for 15 minutes at approximately 1000 x g. Remove serum and perform the assay immediately or aliquot and store samples at -20 °C or -80°C.
- **Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2-8°C within 30 minutes of collection. Store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- **Other biological fluids** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

**NOTE:** Serum, plasma, and cell culture fluid samples to be used within 7 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤2months) or -80°C (≤6months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay, warm up samples to room temperature slowly. DO NOT USE HEAT-TREATED SAMPLES.

Sample Preparation

- Novateinbio is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient amount of samples in advance.
- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
- Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to inaccurate results.

Reagent Preparation

- Bring all kit components and samples to room temperature (18-25 °C) before use.
- **Wash Solution** - Dilute 25 mL of Wash Solution concentrate (20 x) with 475 mL of 2
deionized or distilled water to prepare 500 mL of Wash Solution (1 x).

**Assay procedures**

1. Prepare all the Standards before starting assay procedure (Please read Reagents Preparation). It is recommended that all Standards and Samples should be added in duplicate to the Microtiter Plate.

2. Secure the desired numbers of coated wells in the holder.

3. Take the Standards and agitate gently prior to use. Dilute test samples 1:40 with assay diluent. Then add 100μl of Controls or Samples to the appropriate well of the antigen pre-coated Microtiter Plate.

4. Add 100μl of assay buffer to blank well. Mix well. Cover and incubate the plate for 30 minutes at room temperature.

5. Wash the Microtiter Plate using one of the specified methods indicated below:
   - **Manual Washing**: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with diluted wash solution, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure five times for a total of FIVE washes. After washing, invert plate, and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears. **Note**: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.
   - **Automated Washing**: Wash plate FIVE times with diluted wash solution (350-400μl/well/wash) using an auto washer. After washing, dry the plate as above. It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.

6. Add 100 μl of HRP conjugate into each well and incubate for 30 minutes at room temperature.

7. Wash the plate as described in step 5.

8. Add 100μl of TMB Substrate solution into each well. Cover and incubate for 10 - 30 minutes at room temperature. (Protect from light. Do not over develop).

9. Add 100μl Stop Solution to each well. Mix well.

10. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.
Important notes

• The operation should be carried out in strict accordance with the provided instructions.
• Store the unused strips in a sealed foil bag at 2-8°C.
• Always avoid foaming when mixing or reconstituting protein solutions.
• Pipette reagents and samples into the center of each well, avoid from bubbles.
• The samples should be transferred into the assay wells within 15 minutes of dilution.
• We recommended that all controls, testing samples be tested in duplicate.
• If the blue color develops too shallow after 15 minutes incubation with the substrates, it may be appropriate to extend the incubation time. (Do not over develop)
• Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
• Avoid using the suction head without extensive wash.
• Do not mix the reagents from different batches.
• Stop Solution should be added in the same order of the Substrate solution.
• TMB substrate solution is light sensitive. Avoid prolonged exposure to the light.

Result

• Negative control OD must be less than 0.2 at 450nm.
• Positive control OD must be more than 0.5 at 450nm.
• Cutoff: Test value > 0.2