Schistosoma japonicum IgG ELISA Kit

Cat. No.: DEIA1091
Pkg. Size: 96T

Intended use

The kit takes the Schistosoma japonicum eggs antigen as the solid phase antigen to detect specific antibody against Schistosoma japonicum in serum.

General Description

After Schistosoma japonicum infects an organism, the specific antibody against Schistosoma japonicum arises in serum, whereby detecting the specific antibody against Schistosoma japonicum can be the secondary diagnostic criteria.

Principle Of The Test

This kit employs a solid phase, indirect ELISA assay for detection of IgG antibodies to Schistosoma japonicum in a two-step incubation procedure. Polystyrene microwell strips are pre-coated with purified Schistosoma japonicum antigens. During the first incubation step, Schistosoma japonicum IgG specific antibodies, if present, will be bound to the solid phase pre-coated antigen complexes. The wells are washed to remove unbound serum proteins, and horseradish peroxidase (HRP) labelled anti-IgG antibodies (anti-IgG) conjugate are added. During the second incubation step, these HRP-conjugated antibodies will be bound to any antigen-IgG complexes previously formed and the unbound HRP-conjugate is then removed by washing. Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added to the wells. In presence of the antigen-(IgG)-anti-IgG (HRP) immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for IgG antibodies to Schistosoma japonicum remain colorless.

Reagents And Materials Provided

1. 8× Coated microwell strips: ready to use
2. 1× Conjugate solution: ready to use
3. 1× Washing solution: ready to use
4. 1× Substrate A solution: ready to use
5. 1× Substrate B solution: ready to use
6. 1× Sample dilution: ready to use
7. 1× Stopping solution: ready to use

Warning: Stopping solution irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor.
8. 1× Positive control: ready to use
9. 1× Negative control: ready to use
10. 1× Instruction sheet

Materials Required But Not Supplied

1. Validated microplate reader.
2. Deionized or distilled water.
3. Validated adjustable micropipettes, single and multi-channel.
4. Automatic microtiter plate washer or manual vacuum aspiration equipment.
5. 37 °C incubator.

Storage
Store at 4°C for frequent use, at -20°C for infrequent use. Avoid multiple freeze-thaw cycles.

Assay Steps
Bring all reagents to room temperature (18 - 25°C) before use.
1. Sample preparation
Dilute the patient’s serum with sample dilution at 1:100, e.g. 10 μl serum + 1 ml sample dilution, and mix thoroughly.
2. Adding samples and controls
Leave well A1 for reagent blank. Pipette controls and samples as follows:
100μl negative control and positive control respectively, and 100μl diluted samples each into remaining wells.
Incubation at 37°C for 15 minutes. Discard off contents of the wells and add each well of washing solution, fill all wells with distilled water completely, incubate for 15 seconds and discard off. Perform another 4 washing cycles as above. At the end of the washing step carefully remove remaining fluid by tapping the strips on the tissue paper prior to the next step.
3. Adding conjugate solution
Dispense 2 drops into all wells and incubate at 37°C for 15 minutes. Discard the contents of the wells and wash 5 times as described in step 2.
4. Adding substrates
Dispense one drop of substrate A solution and B solution respectively, incubate at room temperature (37°C) for 10 minutes.
Dispense one drop of stopping solution into all wells. Zero the ELISA microtiter plate reader using the reagent blank well A1.
Measure the absorbance at 450 nm.

Quality Control
1. Observation with naked eyes before adding stopping solution
Negative: No apparent blue appears in the sample wells. This indicates that no cysticercus antibody has been detected.
Positive: Apparent blue appears in the sample wells. This indicates that the sample contains cysticercus antibody.
Invalid: If no blue appears in the positive control well or apparent blue is observed in the negative control well, this indicates a possible error in performing the test. The test should be repeated using a new kit.
2. Judgment by microtiter plate reader
Cut-off value = 2.1×A- (absorbance of negative control)

Reproducibility
Intra-Assay: CV<10%
Inter-Assay: CV<10%

Precautions
1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. CAUTION - CRITICAL STEP: Allow the reagents and samples to stabilize at room temperature(18-30°C) before use. Shake reagent gently before, and return to 2-8°C immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
7. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.
8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.
10. The use of automatic pipettes is recommended.
11. Assure that the incubation temperature is 37°C inside the incubator.
12. When adding samples, avoid touching the well’s bottom with the pipette tip.
13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
14. If using fully automated microplate processing system, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.