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User's Manual

Schistosoma japonicum IgM ELISA Kit

REF **DEIA1092**

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.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

For the qualitative determination of IgM class antibodies against Schistosoma japonicum in human serum and plasma.

Principles of Testing

This kit employs solid phase, indirect ELISA assay for detection of IgM antibodies to Schistosoma japonicum in two-step incubation procedure. Polystyrene microwell strips are pre-coated with purified Schistosoma japonicum antigens. During the first incubation step, Schistosoma japonicum IgM 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-gG antibodies (anti-IgM) conjugate are added. During the second incubation step, these HRP-conjugated antibodies will be bound to any antigen-IgM 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-(IgM)-anti-IgM (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 IgM antibodies to Schistosoma japonicum remain colorless.

Reagents And Materials Provided

1. Coated microtiter strips: 96 wells
2. 1× Conjugate solution: 6 mL x 1vial
3. 10× Washing solution: 100 mL x 1vial
4. 1× Substrate A solution: 6 mL x 1vial
5. 1× Substrate B solution: 6 mL x 1vial
6. 1× Sample dilution: 100 mL x 1vial
7. 1× Stopping solution: 6 mL x 1vial
8. 1× Positive control: 0.8 mL x 1vial
9. 1× Negative control: 0.8 mL x 1vial
10. Valve bag: 1
11. Microseal: 1
12. Instruction sheet: 1

Materials Required But Not Supplied

1. Validated microplate reader (450nm and 630nm).
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 2-8°C, avoid light. Do not use the kit beyond the expiration date.

Specimen Collection And Preparation

Serum Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g, 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles. Centrifuge the sample again after thawing before the assay.

Assay Procedure

Bring all reagents to room temperature (18 - 25°C) before use.

1. Wash Buffer (1x)

If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 100 mL of Wash Buffer Concentrate (10 x) into 900 mL deionized or distilled water to prepare 1000 mL of Wash Buffer (1 x).

2. Sample preparation

Dilute the patient's serum with sample dilution at 1:100, e.g. 5 µl serum + 495 µl sample dilution, and mix thoroughly.

3. Adding samples and controls

Leave well A1 for reagent blank. Pipette controls and samples as follows:

100 µl negative control each of 2 wellls and 100µl positive control each of 2 wellls respectively, and 100µl diluted samples each into remaining wells. Incubation at 37°C for 30 minutes in dark place.

4. Discard off contents of the wells and fill all wells with washing solution (1 x), incubate for 60 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.
5. Adding conjugate solution

Dispense 1 drop into all wells and incubate at 37°C for 30 minutes. Discard the contents of the wells and wash 5 times as described in step 4.

6. Adding substrates

Dispense one drop of substrate A solution and B solution respectively, mix thoroughly and incubate at room temperature (37°C) for 10 minutes in dark place.

7. 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 (and 630nm as reference wavelength).

Interpretation Of Results

Positive result: Absorbance of Sample> COV

Negative result: Absorbance of Sample < COV

Reference Values

1. Observation with naked eyes before adding stopping solution

Negative: No apparent blue appears in the sample wells and OD450<0.10. Invalid when OD450>0.10.

Positive: Apparent blue appears in the sample wells and OD450>0.50. Invalid when OD450<0.50.

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. Judgement by microtiter plate reader

Cut-off value (COV) = 2.1×A

A: absorbance of negative control (absorbance value of negative control should be regarded as 0.07 if it is below 0.07).

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 assay.
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.