DRG® Rubella IgM (EIA-1801)

Revised 24 Jan. 2011 rm (Vers. 4.1) **RUO**

ENZYME IMMUNOASSAY FOR THE DETECTION OF IgM ANTIBODIES TO RUBELLA VIRUS IN HUMAN SERUM

THIS KIT IS INTENDED FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PRINCIPLE OF THE TEST

Purified Rubella antigen is coated on the surface of microwells. Diluted serum is added to the wells, and the Rubella IgM- specific antibody, if present, binds to the antigen during incubation. After washing the wells to remove unbound sample, antibody to human IgM conjugated with horseradish peroxidase (HRP) is added and incubated at 37°C for 30 minutes. Unbound conjugate is removed by a subsequent washing step. A solution of TMB Reagent is then added to the microwells. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgM-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

REAGENTS

Materials provided with the kit:

- Microtiter Wells: purified *Rubella* antigen coated wells (12 x 8 wells).
- Enzyme Conjugate Reagent (red color): Red cap. 1 vial (12 ml).
- Sample Diluent (blue color): Natural cap. 1 bottle (22 ml).
- Negative Control: Range stated on label. Natural cap (100 µL/vial).
- Cut-off Calibrator: Yellow cap. *Rubella IgM* Index = 1 (100 µL/vial).
- Positive Control: Range stated on label. Red cap. (100 µL/vial).
- Wash Buffer Concentrate (20x): Natural cap. 1 bottle (50 ml).
- TMB Regaent (One-Step): 1 vial (11 ml).
- Stop Solution: 1N HCl, Natural cap. 1 vial (11 ml).

STORAGE OF TEST KITS AND INSTRUMENTATION

1. Store the kit at 2-8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

Warnings and Precautions

1. **POTENTIAL biohazardous materials**: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not pipette reagents by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.
5. For Research Use Only.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25 °C) before use.
2. Dilute 1 volume of Wash Buffer (20x) with 19 volumes of distilled water. For example, dilute 50 ml of Wash Buffer (20x) into distilled water to prepare 1000 ml of Wash Buffer (1x). Wash Buffer is stable for 1 month at 2-8°C. Mix well before use.

ASSAY PROCEDURE

1. Place the desired number of coated wells into the holder.
2. Prepare 1:40 dilution of test samples, Negative Control, Positive Control, and Calibrator by adding 5 µl of the sample to 200 µl of Sample Diluent. Mix well.
3. Dispense 100 µl of diluted sera, Calibrator, and Controls into the appropriate wells. For the reagent blank, dispense 100 µl Sample Diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well.
4. Incubate at 37°C for 30 minutes.
5. At the end of incubation period, remove liquid from all wells. *Rinse and flick the microtiter wells 5 times with diluted Wash Buffer (1x).*
6. Dispense 100 µl of Enzyme Conjugate to each well. Mix gently for 10 seconds.
7. Incubate at 37°C for 30 minutes.
8. Remove Enzyme Conjugate from all wells. *Rinse and flick the microtiter wells 5 times with diluted Wash Buffer (1x).*
9. Dispense 100 µl of TMB Reagent into each well. Mix gently for 10 seconds.
10. Incubate at 37°C for 15 minutes.
11. Add 100 µl of Stop Solution (1N HCl) to stop reaction.
12. Mix gently for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***

Note: Make sure there are no air bubbles in each well before reading.

13. Read O.D. at 450nm ***within 15 minutes*** with a microwell reader.

CALCULATION OF CUT-OFF RESULTS

1. Calculate the mean of duplicate cut-off calibrator value x_c.
2. Calculate the mean of duplicate positive control (x_h), negative control (x_l) and samples(x_s).
3. Calculate the Rubella IgM Index of each determination by dividing the mean values of each sample (x) by calibrator mean value, x_c.

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