RUBELLA IgM Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIARUB2	RUBELLA IgM Elisa	96 Tests

Intended Use:

The RUBELLA IgM Elisa is intended to be used for the detection of IgM antibodies to Rubella in Human serum. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

The Rubella virus is the causative virus of the congenital rubella syndrome when infection occurs during the first weeks of pregnancy. Congenital rubella syndrome is a disease in which children suffer from hearing impairments, eye and heart defects and other lifelong disabilities, including autism, diabetes mellitus and thyroid dysfunction. This Elisa assay uses microplates coated with Rubella antigen. Upon presentation of the sample and if it contains Rubella IgM antibodies, these antibodies combine with the antigen coated onto the microplate. HRP conjugated human anti-IgM is then added which binds where the antigen-(RV IgM)-antibody complexes have formed and subsequently a blue colour forms when a substrate is added. The reaction is stopped using an acid solution that changes the colour to yellow. The use of a cut off control and a positive control provides the necessary absorbances to classify patient samples as being positive or negative.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	One 96 well microplate coated with Rubella antigen. Stable when stored at 2-8°C till the expiry date.
Negative Control	1x1ml	Tris Buffer containing salts and preservatives. Stable when stored at 2-8°C till the expiry date. Once opened use within 60 days.
Positive Control	1x1ml	Tris Buffer containing Rubella IgM, salts and preservatives. Stable when stored at 2-8°C till the expiry date. Once opened use within 60 days.
HRP Conjugate	1x6.5ml	1vial containing anti-human IgM conjugated with HRP. Store at 2-8°C. Once opened use within 60 days.
Sample Diluent	1x11ml	Calf Serum for diluting samples. Store at 2-8°C. Once opened use within 60 days.
Wash Solution Concentrate	1x20ml	One vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.
Substrate A	1x7ml	One bottle containing Hydrogen peroxide in buffer. Store at 2-8°C.
Substrate B	1x4ml	One bottle containing TMB in buffer. Store at 2-8°C.
Stop Solution	1x6ml	One bottle containing H2SO4. Store at 2-8°C.

Plastic Sealable bag, IFU and plate covers.

NOTE 1: Do not use reagents beyond the kit expiration date.

NOTE 2: Avoid extended exposure to heat and light. Opened reagents are stable for 60 days when stored at 2-8°C. Kit and component stability are identified on the label.

NOTE 3: Above reagents are for single 96 well microplate.

Materials required but not provided:

Distilled water, Timer, Micropipettes, Incubator, Microplate Reader and Microplate washer.

Specimen Collection, Storage and Stability:

Serum is the sample of choice. Collect serum samples in accordance with correct medical practices. Ensure that the samples are clear and do not have suspended particles or sediments. Blood should be collected in plain red top venepuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of 5 days. If the specimens cannot be assayed within this time, the samples may be stored in temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.05ml of the specimen is required.

Procedure:

Reagent preparation:

1. **Wash Buffer**: Dilute wash buffer concentrate in the ratio of 1:40. (i.e., for every ml of the concentrate add 40 ml of distilled water) Store diluted buffer at 2-30°C for up to 60 days.

TEST PROCEDURE:

Before proceeding with the assay, being all reagents and controls to room temperature (20-27°C)

STEP 1

Preparation: Format the microplate wells for each blank well, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminium bag, seal and store at 2-8°C.

STEP 2

Addition of Sample Diluent: Add Sample Diluent 100ul (2drops) into the appropriate wells except the blank well, positive control well and the negative control well.

STEP 3

Addition of Sample: Add 10ul of the sample to the wells mixing it with the sample diluent by repeatedly pipetting in and out of the well until the liquid turns blue. Dispense 100ul of Negative and the Positive control to their respective wells. Do not dispense anything into the blank well.

STEP 4

Incubation: Swirl the microplate gently for 30 seconds to mix and cover the plate with the plate cover and incubate for 20 minutes at 37°C.

STEP 5

Washing: At the end of the incubation period, remove and discard the plate cover and the contents of the microwell. Wash each well 5 times with diluted washing buffer of 350ul with a soak time of 20 seconds each time. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer. If a squeeze bottle is used (instead of an automatic microplate washer) then fill each well by depressing the container (avoiding air bubbles) to dispense the wash.

STEP 6

Addition of HRP Conjugate: Add 1 drop (50ul) of the HRP Conjugate to each well except the blank well. Gently mix the contents of the well.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 20 minutes at 37°C. Ensure that the incubation is done in the dark.

STEP 8

Washing: At the end of the incubation period, remove and discard the plate cover and the contents of the microwell. Wash each well 5 times with diluted washing buffer of 350ul with a soak time of 20 seconds each time. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer. If a squeeze bottle is used (instead of an automatic microplate washer) then fill each well by depressing the container (avoiding air bubbles) to dispense the wash.

STEP 9

Addition of Substrate: Add 1 drop of Substrate A (50ul) and one drop of Substrate B (30ul) into all wells except the blank well. Incubate at 37°C for 10 minutes

STEP 10

Stopping the Reaction: Add 1 drop (50ul) of the Stop solution into each well and mix gently. Shake the plate gently for 15-20 seconds to mix, till the solution changes to yellow from blue.

STEP 11

Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader. Note down the absorbances. Read the results within 30 minutes of addition of the stop solution. (when using a reader without the facility of a secondary wavelength use the blank well absorbance and subtract the absorbance from the negative, positive and the sample absorbances).

Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.

CUT OFF OD: 2.1 x Negative Control OD.

IF THE OD VALUE OF THE NEGATIVE CONTROL IS LOWER THAN 0.05 CALCULATE CUT OFF USING 0.05 ABSORBANCE. IF THE OD VALUE OF THE NEGATIVE CONTROL IS GREATER THAN 0.05 USE THE ACTUAL ABSORBANCE.

Interpretation:

Positive for Rubella IgM: sample OD is >/= Cut off OD

Negative for Rubella IgM: sample OD is < Cut off OD

Validation:

Negative OD/Cut-off OD (Nc/CO) should be between 0.1 and 0.8

Positive OD/Cut-off OD (Pc/CO) should be between 4.5 and 18.0

Performance Characteristics:

Specificity: 100% and Sensitivity: 90%.

Interferences: there is no interference from RF up to 120 IU/ml, bilirubin up to 80 mg/dl, triglycerides up to 3000 mg/dl and EB antibody up to 100 U/ml. Haemoglobin of 2000 mg/dl gives false positive results, do not use haemolysed samples.

Notes:

1. Do not mix reagents from different batches of reagents.
2. Gently mix the reagents before use.
3. Wash Buffer concentrate may have crystals under different temperature conditions. Ensure that the wash buffer concentrate is homogeneous before use.
4. Keep unused microwells in the original bag.
5. Do not use haemolytic, lipaemic, bacteria contaminated or heat inactivated samples.
6. Assay performance characteristics have not been established for visual estimations.
7. Caution should be used when evaluating samples obtained from immunosuppressed patients.
8. Run validity is determined through the performance of the positive and negative controls as well as the blank.
9. Positive and negative controls are intended to monitor reagent failure and not ensure precision at the assay cut off levels.
10. NCCLS document C24-A3: Statistical Quality Control for Quantitative measurements can be used as a guidance document for QC practices.

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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