Rubella IgM ELISA Kit

Qualitative assay for anti-Rubella IgM antibodies
Product code GD83
96 tests
For *in vitro* research use only

270808

1. Intended use

The Rubella IgM kit is a rapid ELISA designed for the qualitative detection of IgM antibodies to Rubella virus in human serum. The assay is designed to evaluate serologic evidence of active or recent infection with Rubella virus, and is for research use only. Plasma samples may also be used.

2. Introduction

Rubella (German Measles) is a common and usually benign contagious disease of children and young adults. The primary medical significance comes from its teratogenic effects when contracted by childbearing women. Maternal infection, especially during the first trimester of pregnancy, can result in a range of congenital birth defects including deafness, cataracts, diabetes and cardiac and bone abnormalities. Because of the serious complications of the disease, it is important to determine the immune status of women of child bearing age, pregnant women, and individuals who may have close contact with them.

The presence of circulating maternal antibody indicates immunity to Rubella and virtually excludes the possibility of transmission of Rubella to the foetus. Acute Rubella infection can be confirmed by simultaneously testing paired acute and convalescent sera and looking for seroconversion, or by detecting Rubella specific IgM. The presence of Rubella specific IgM in the neonate or the persistence of a high titre of IgG antibody for longer than 6 months confirms a diagnosis of congenital Rubella. Pregnant women with current Rubella infection should be counselled on the consequences of congenital infection

3. Principle of the test

Microplates are coated with a highly purified viral envelope spike protein. This protein is comprised of antigens E_1 and E_2 in the native configuration and these antigens give greater specificity in the detection of Rubella IgM antibodies. Test sera are diluted with the sample diluent provided. Anti-human IgG is added to the sample diluent sample to eliminate the possibility of interference by antigen-specific IgG and rheumatoid factor, if present. Diluted serum or plasma specimens (1:100) are incubated for 20 minutes to allow specific antibodies to Rubella to bind to the antigen-coated wells. After washing away unbound antibodies and other serum constituents, Rubella specific IgM is detected using rabbit anti-human IgM conjugated to horseradish peroxidase. After 20 minutes incubation, unbound conjugate is removed by washing, and TMB enzyme substrate is added for 10 minutes. A blue colour develops if antibodies to Rubella are present. Addition of stop solution gives a yellow colour and the optical densities of controls, 10 U/ml standard and samples are measured using a microplate reader.

4. Materials included in the Kit

- **Microplate** 96 wells in 12 X 8 break-apart strips, pre-coated with inactivated Rubella viral envelope spike protein.
- **Reagent 1**: Sample diluent 46 ml, (blue). Read the instructions before use.
- **IgG absorbent:** Anti-human IgG, 3X3.5ml. Read the instructions before use.
- **Reagent 2: Wash Buffer** 100mM Tris-buffered saline with detergent, pH 7.2, 100 ml, **concent**rate (x10)
- **Reagent 3:** Conjugate (peroxidase conjugated rabbit anti-human IgM), 12 ml, (green). Ready to use
- **Reagent 4:** TMB substrate, 12 ml. Ready to use
- **Reagent 5:** Stop solution, 12 ml. Ready to use
- **Positive control:** (red), 1ml liquid. Ready to use.
- **Standard:** 10 U/ml, (yellow), 1ml liquid. Ready to use.
- **Negative control:** (green), 1ml liquid. Ready to use.
- **Instructions for use**

5. Other equipment required

10mm X 60mm tubes for dilution, pipettes 10µl, 100µl, 1000µl; repeating dispenser 100µl, microplate reader with 450nm filter, microplate washing device. Distilled or de-ionised water, general laboratory apparatus.

6. Storage and precautions

On arrival, store the kit at 2 - 8ºC. Once opened the kit is stable for three months (or until its expiry date if less than three months). It is important to protect the unused wells from excess moisture. Do not use kits beyond their expiry date.

The assay 10 U/ml standard and controls are manufactured from dilute non-infectious human serum. Normal clinical laboratory safety procedures should be maintained at all times. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.

The stop solution contains 0.24M sulphuric acid and is non-corrosive.

7. Samples

Only freshly drawn and properly refrigerated sera or plasma should be used in this assay. Avoid haemolysed, lipemic or bacterial contaminated sera. Sera should be stored at 2-8ºC for no longer than 5 days. If delay in testing is anticipated, store test sera at –20ºC. Avoid multiple freeze-thaw cycles.

8. Method

Ensure that all materials are at room temperature before beginning the procedure. We recommend that the 10 U/ml standard and the controls be always run in duplicate. Samples may be run singly or in duplicate.

1. Assemble the number of strips required for the assay.

2. Prepare only sufficient IgG-absorbent-containing sample diluent for the number of samples to be tested. Add one part **IgG absorbent** to 4 parts of **Reagent 1 Sample Diluent** as shown in the examples below and mix thoroughly. Discard any unused IgG-absorbent-containing diluent.

Approx Nr of samples	Volume of sample diluent (ml)	Volume of IgG Absorbent (ml)
24	10	2.5
48	20	5.0
72	30	7.5
96	40	10.0

3. Dilute patient samples 1:100 (e.g. 5µl serum plus 0.5 ml diluent). It is important to dispense all samples and controls into the wells without delay. Therefore ensure that all samples are ready to dispense.

4. Dispense 100 µl of the negative control, the 10 U/ml standard, the positive control and the diluted patient sample into the wells.

5. Incubate for **20** minutes at room temperature. During all incubations, avoid direct sunlight and close proximity to any heat sources.

6. Dilute the Wash Buffer (**Reagent 2)** 1: 9 in distilled water to make sufficient buffer for the assay run e.g. add 50ml wash buffer concentrate to 450ml water. The diluted wash buffer is stable for two months at 2 - 8ºC.

7. After 20 minutes, decant or aspirate the well contents and wash the wells 3 times using an automatic plate washer or the manual wash procedure (see below). Careful washing

is the key to good results. Blot the wells on absorbent paper before proceeding. **Do not allow the wells to dry out.**

Manual Wash Procedure:
Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with wash buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process two more times.

8. Dispense 100µl of Conjugate (**Reagent 3)** into each well. This reagent is colour coded green. Keep all pipettes and other equipment used for Conjugate completely separate from the substrate reagent! Incubate the wells for **20** minutes in the incubation bag at room temperature.

9. After 20 minutes, discard the well contents and carefully wash the wells with wash buffer. Repeat the washing stage three more times. Ensure that the wells are completely washed. Blot the microplate on absorbent paper to remove final drops of wash fluid. **Do not allow the wells to dry out.**

10. Using a repeating dispenser, rapidly dispense 100µl of TMB substrate (**Reagent 4)** into each well. Incubate the plate for **10** minutes.

11. Add 100µl of Stop solution (**Reagent 5)** to each well. To allow equal reaction times, the Stop solution should be added to the wells in the same order as the TMB substrate.

12. Read the optical density in a microplate reader within 10 minutes.

9. Quality control

The expected optical density values for the negative and positive controls and the 10 U/ml standard are given on the certificate included in the kit.

10. Interpretation

Negative samples: OD < OD of 10 U/ml standard
Positive samples: OD >/= OD of 10 U/ml standard

1. A negative result indicates no current or recent infection with Rubella virus. In the absence of a previous infection, such individuals are presumed susceptible to primary infection. However, specimens taken too early during a primary infection may not have detectable levels of IgM antibody. If a primary infection is suspected, another specimen should be taken in 8-14 days and tested concurrently in the same assay with the original specimen to identify seroconversion.

2. A positive result indicates current or recent infection with Rubella virus. Individuals with current infection are considered to be at risk of transmitting Rubella virus infection.

11. Limitations

1. Results of the Genesis Diagnostics Rubella IgM test should be interpreted together with the patient's clinical condition and results of other diagnostic procedures.
2. Rubella virus specific IgG antibody may compete with IgM for binding sites and cause false negative results. Rheumatoid factor, if present along with specific IgG, will cause false positive results. The sample diluent contains anti-human IgG and effectively removes IgG from the test specimen significantly reducing the possibility of false negative and false positive results.
3. Heterotypic IgM antibody responses may occur in patients infected with Epstein-Barr virus, and sera from patients with infectious mononucleosis may have false positive results in the Rubella IgM ELISA.
4. The Genesis Diagnostics IgM assay cannot distinguish between vaccine-induced antibody and antibody resulting from natural infection.
5. False positive results may be obtained from patients with autoimmune disease.
6. The performance of the Genesis Diagnostics Rubella IgM assay has not been validated using neonatal samples.

12. Expected Values

During a primary infection with Rubella virus, IgM specific antibodies become detectable within five days after onset of rash. IgM specific antibodies remain detectable for one month but may persist for longer than 6 months in some patients.

13. Performance characteristics

Comparative study:

Results for specimens obtained from UKNEQAS were 100% concordant with the defined responses for the samples.

14. Assay characteristics

Within Assay Imprecision < 12%
Between Assay Imprecision < 12%

Method Summary

- Mix IgG absorbent and Sample Diluent 1:4 and dilute all samples 1:100
- Dispense 100µl of 10 U/ml standard, each control and diluted sample into the microplate wells
- Incubate for **20** minutes at room temperature.
- *Wash the wells three times*
- Dispense 100µl of Conjugate **(Reagent 3)** into each well
- Incubate at room temperature for **20** minutes
- *Wash the wells four times*
- Add 100µl of TMB substrate **(Reagent 4)** to each well
- Incubate at room temperature for **10** minutes
- Add 100µl Stop solution **(Reagent 5)** to each well
- Read the optical density at 450nm

Further Reading

Vaheri A and Salonen EM: Evaluation of solid-phase enzyme- immunoassay procedure for in immunity surveys and diagnosis of rubella. J Med Virol 5: 171-181, 1980.

Enders G et al: Comparison of various serological methods and diagnostic kits for the detection of acute, recent, and previous rubella infection, vaccination, and congenital infections. J Med Virol 16: 219-232, 1985.