

### 11. Assay Procedure Continued...

- After 20 minutes, discard the well contents and carefully wash the wells 4 times with Wash Buffer. Ensure that the wells are empty but do not allow to dry out.
- Using a repeating dispenser, rapidly dispense 100µl of TMB Substrate (**Reagent 4**) into each well. Incubate the plate for **10 minutes**.
- 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.
- Read the optical density (OD) of each well at 450nm in a microplate reader within 10 minutes. A 620nm filter may be used as a reference wavelength.

### 12. Quality Control

Quality control data is supplied on the lot-specific QC certificate included in the kit. Controls are intended to monitor for substantial reagent failure. Any well positive by spectrophotometer but without visible colour should be cleaned on the underside and re-read. If OD-values below zero are observed, the wavelengths used should be verified, the reader re-blanked to air and the measurements repeated.

### 13. Interpretation of Results

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

- 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.
- 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.

### 14. Limitations of the Procedure

- Results of the Genesis Diagnostics Rubella IgM test should be interpreted together with the patient's clinical condition and results of other diagnostic procedures.
- 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.
- 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.
- The Genesis Diagnostics IgM assay cannot distinguish between vaccine-induced antibody and antibody resulting from natural infection.

- False positive results may be obtained from patients with autoimmune disease.
- The performance of the Genesis Diagnostics Rubella IgM assay has not been validated using neonatal samples.
- 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.

### 15. Performance Characteristics

#### Comparative Study

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

### 16. Reproducibility

#### Within Assay Precision

CV%: <12%

#### Between Assay Precision

CV%: <12%

### 17. Method Summary

- Mix IgG absorbent and Sample Diluent 1:4 and dilute all samples 1:100
- Dispense 100µl of the 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

### 18. 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.

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## Rubella IgM ELISA Kit

Qualitative assay for anti-Rubella IgM antibodies

Product Code: GD083

For Research Use Only. Not for use in diagnostic procedures

Not for sale or use in the EU

### 1. 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** 10mM Tris-buffered saline, pH 7.2 with antimicrobial agent, 46ml, (blue). Read the instructions before use.
- IgG absorbent:** Anti-human IgG, 3 x 3.5ml. Read the instructions before use.
- Reagent 2: Wash Buffer** 100mM Tris-buffered saline with detergent, pH 7.2, 100 ml, **concentrate** (x10)
- Reagent 3: Conjugate** goat anti-human IgM (green) conjugated to horseradish peroxidase in protein stabilising solution and antimicrobial agent, 12ml, ready to use
- Reagent 4: TMB Substrate** aqueous solution of TMB and hydrogen peroxide, 12 ml, ready to use
- Reagent 5: Stop Solution** 0.25M sulphuric acid, 12 ml, ready to use
- Standards:** 10 U/ml (yellow), 1ml of 10mM Tris-buffered saline with human serum IgM antibodies Rubella, ready to use
- Positive control:** 1ml of 10mM Tris-buffered saline containing human serum antibodies to Rubella, 100 U/ml, (red), ready to use.
- Negative control:** 1ml of 10mM Tris-buffered saline containing normal human serum, (green), ready to use.
- Instructions for use**

## 2. Other Equipment Required

Test tubes for dilution • graduated cylinder for preparing wash buffer • precision pipettes and disposable tips to deliver 10µl, 100µl, 1ml • EIA microplate washer or multi-channel pipette or wash bottle • distilled or de-ionised water • absorbent paper • EIA microplate reader with 450nm and optional 620nm reference filter. Alternatively, a suitable, self-validated automated system may be used.

Instrumentation, whether manual or automated, should meet the following criteria: pipettes with better than 3% imprecision with no carry over between pipetting steps; microplate washers should remove 99% of fluid; automated machines should minimise time between washing and adding the next reagent.

## 3. Intended Use

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

## 4. Explanation of the Test

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.

## 5. Principle of the Test

Microplates are coated with a highly purified viral envelope spike protein. This protein is comprised of antigens E<sub>1</sub> and E<sub>2</sub> 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.

## 6. Safety Precautions

1. Only experienced laboratory personnel should use this test. The test protocol must be followed strictly.
2. CAUTION: the device contains material of human and animal origin and should be handled as a potential transmitter of diseases. All human source material used in the preparation of standards and control for this product have been tested and found negative by ELISA for antibodies to HIV, HbsAg and HCV. No test method, however, can offer complete assurance that infectious agents are absent. Therefore, all reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
3. Reagents of this kit contain antimicrobial agents and the Substrate solution contains 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
4. The Stop Solution contains 0.25M sulphuric acid. Avoid contact with skin and eyes. Rinse immediately with plenty of water if contact occurs.
5. Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Dispose of plates and specimens as clinical waste. Any unused reagents should be flushed away with copious amounts of water. Disposal must be performed in accordance with local legislation.

## 7. Technical Precautions

1. Strips and solutions should not be used if the foil bag is damaged or liquids have leaked.
2. Allow all reagents and the microplate to reach room temperature before use. Ensure that the microplate foil bag containing any unused strips is well sealed and contains the desiccant to avoid moisture. Store at 2 – 8°C after use.
3. Include the Positive and Negative Control in every test run to monitor for reagent stability and correct assay performance.
4. Strictly observe the indicated incubation times and temperature.
5. When automating, consider excess volumes required for setting up the instrument and dead volume of robot pipette
6. Ensure that no cross-contamination occurs between wells. Keep all pipettes and other equipment used for Conjugate completely separate from the TMB Substrate reagent.
7. When pipetting Conjugate or TMB Substrate, aliquots for the required numbers of wells should be taken to avoid multiple entry of pipette tips into the reagent bottles. Never pour unused reagents back into the original bottles.
8. Do not allow microwells to dry between incubation steps.
9. Strictly follow the described wash procedure. Insufficient washing may cause high background signal.
10. Avoid direct sunlight and exposure to heat sources during all incubation steps.
11. Replace colour-coded caps on their correct vials to avoid cross-contamination
12. It is important to dispense all samples and controls into the wells without delay. Therefore ensure that all samples are ready to dispense.

## 8. Shelf Life and Storage Conditions

On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for 3 months (or until its expiry date if less than 3 months). Do not use kits beyond their expiry date. Do not freeze any kit component. The diluted Wash Buffer has a shelf life of 3 months if stored in a closed bottle at 2 - 8°C.

## 9. Specimen Collection and Storage

Serum and plasma samples may be used and should be stored at -20°C for long-term storage. Frozen samples must be mixed well after thawing and prior to testing. Repeated freezing and thawing can affect results. Addition of preservatives to the serum sample may adversely affect the results. Microbially contaminated, heat-treated or specimens containing particulate matter should not be used. Grossly haemolysed, icteric or lipaemic specimens should be avoided.

## 10. Preparation of Reagents

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

Approx. # of Samples	Vol. of Sample Diluent (ml)	Vol. of IgG Adsorbent (ml)
24	10	2.5
48	20	5.0
72	30	7.5
96	40	10.0

2. 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.

## 11. Assay Procedure

1. Dilute patient samples 1:100 (e.g. 5µl serum plus 0.5 ml IgG-adsorbent-containing sample diluent). It is important to dispense all samples, standards and controls into the wells without delay. Therefore ensure that all samples are ready to dispense.
2. Assemble the number of strips required for the assay.
3. For qualitative assays, dispense 100 µl of the negative control, the 10 U/ml standard, the positive control and the diluted patient sample into the wells.
4. Incubate for **20 minutes** at room temperature. During all incubations, avoid direct sunlight and close proximity to any heat sources.
5. 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 2 more times.

6. Dispense 100µl of Conjugate (**Reagent 3**) into each well. Incubate the wells for **20 minutes** at room temperature.