The Rubella IgG EIA Test Kit is an enzyme immunoassay (EIA) for the qualitative and quantitative detection of IgG antibodies to Rubella in human serum or plasma. It is intended as an aid in the diagnosis of possible Rubella infection.

### Materials Provided

**Rubella IgG Microwell Plate**
- 96 wells/plate

**Materials Required But Not Provided**

- Calibrated micropipettes with disposable tips capable of dispensing 5, 50 and 100 μL
- Sodium hypochlorite solution for disinfecting work surface
- Vortex mixer for specimen mixing (optional)
- Vortex mixer for specimen mixing (optional)

### Principle

The Rubella IgG EIA Test Kit is a solid-phase enzyme immunoassay based on indirect principle for the detection of IgG antibodies to Rubella virus in human serum or plasma. The microwell plate is coated with Rubella antigen. During the test, the specimen is incubated with the microwell plate to form an antigen-antibody complex. The unbound specimen is removed, and the bound antigen-antibody complexes are washed and identified with specific enzyme-labeled antibody. An enzyme substrate is added, and the amount of enzyme activity is measured using a plate reader.

### Summary

Rubella is a small spherical enveloped RNA virus belonging to Togavirus family. Most commonly known as the German or 3-day measles, the Rubella virus is spread through droplet infection resulting in mild cutaneous rash followed by fever and lymphadenopathy in young adults. In childhood, the infection is self-limited, benign disease characterized by low-grade fever, headache, lymphadenopathy, arthralgia, and conjunctivitis. However, infection during pregnancy particularly in the first trimester can lead to spontaneous abortion, intrauterine infection causing fetal death, or congenital abnormalities. Congenital rubella disease is a common complication of infection occurs and may result in severe sequelae including deafness, ocular problems including cataracts and glaucoma, congenital heart disease and mental retardation. 1 Rubella IgM antibodies against rubella are first produced during detectable levels within 2-3 days of exposure. The IgM antibodies remain detectable for the next 4-8 weeks. Diagnosis of active or recent infection may be obtained by presence of IgM antibodies and early specimens. After the acute phase of the illness, the IgM antibodies will peak 14-21 days later which will persist for variable levels for life. 1, 2 The presence of IgG antibodies to Rubella is indicative of previous infection and presumptive immunity.

### Materials Required But Not Provided

- Calibrated micropipettes with disposable tips capable of dispensing 5, 50 and 100 μL
- Sodium hypochlorite solution for disinfecting work surface
- Vortex mixer for specimen mixing (optional)
- Vortex mixer for specimen mixing (optional)

### Precautions

- Follow the wash procedure to ensure optimum assay performance.
- Do not store Stop Solution in a shallow dish or return it to the original bottle after use. Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the color reaction may be inhibited.
- Some components of this kit are derived from human blood derivatives which were found to be non-reactive for the HIV-1/HIV-2/HIV-O, Syphilis and HCV antibodies, as well as HBsAg. But no evidence has been reported on the reactivity for the HIV-1/HIV-2/HIV-O, Syphilis and HCV antibodies, as well as HBsAg. But no evidence has been reported on the reactivity for the HBsAg, anti-HBc and Anti-HBs. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.

### DIRECTIONS FOR USE

1. Unopened test kits should be stored at 2-8°C. Exposed test kits should be stable through the expiration date printed on the box if stored between 2-8°C. Once opened, all reagents are stable for up to 3 months after the first opening date if stored between 2-8°C. Return reagents to the refrigerator immediately after use.

2. Allow the sealed pouch to reach room temperature before opening the pouch and removing the reagents from the pouch. Do not expose reagents to temperatures above 30°C or below 2°C. Store reagents at room temperature after opening the pouch.

3. Separate serum or plasma from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Specimen with extensive particulate should be clarified by centrifugation prior to use. Do not use specimens with fibrin particles or contaminated with microbial growth.

4. Follow the wash procedure to ensure optimum assay performance.

5. Do not mix reagents from other kits with different lot numbers. Avoid cross contamination between reagents to ensure valid test results.

6. Follow the wash procedure to ensure optimum assay performance.

7. Do not use pipets that have been used for dispensing reagents containing highly concentrated reagents. Do not touch the bottom of the wells with pipettes. Do not touch the bottom of the microwell plate.

8. Do not store unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C.

### SPECIFIC COLLECTION AND PREPARATION

- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.

- Do not mix reagents from other kits with different lot numbers. Avoid cross contamination between reagents to ensure valid test results.

- Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the color reaction may be inhibited.

### REAGENT COMPONENTS

<table>
<thead>
<tr>
<th>No.</th>
<th>Reagent</th>
<th>Description</th>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rubella IgG Microwell Plate coated with purified Rubella antigens</td>
<td>96 wells/plate</td>
<td>96 wells/plate</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Rubella IgG Conjugate</td>
<td>Anti-human IgG antibody bound to HRP</td>
<td>0.1% ProClin™ 300</td>
<td>1 x 12 mL</td>
</tr>
<tr>
<td>3</td>
<td>Rubella IgG Antibody Standard 1</td>
<td>Calibrator 1</td>
<td>0.1% ProClin™ 300</td>
<td>5 x 8 mL</td>
</tr>
<tr>
<td>4</td>
<td>Rubella IgG Antibody Standard 2</td>
<td>Calibrator 2</td>
<td>0.1% ProClin™ 300</td>
<td>5 x 8 mL</td>
</tr>
<tr>
<td>5</td>
<td>Rubella IgG Antibody Standard 3</td>
<td>Calibrator 3</td>
<td>0.1% ProClin™ 300</td>
<td>5 x 8 mL</td>
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<tr>
<td>6</td>
<td>Rubella IgG Antibody Standard 4</td>
<td>Calibrator 4</td>
<td>0.1% ProClin™ 300</td>
<td>5 x 8 mL</td>
</tr>
</tbody>
</table>

### MATERIAlS REQUIRED BUT NOT PROVIDED

- Calibrated micropipettes with disposable tips capable of dispensing 5, 50 and 100 μL
- Sodium hypochlorite solution for disinfecting work surface
- Vortex mixer for specimen mixing (optional)
- Vortex mixer for specimen mixing (optional)

### Step 1

- Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25 in a clean, dry and sterile bottle containing the concentrated wash buffer in a graduated cylinder and 88 μL of distilled water to 1250 μL, for 96 wells/plate testing, or 525 μL for 48 wells/plate. Working Washing Buffer is stable for 2 weeks at 15°C. Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the color reaction may be inhibited.

### Step 2

- Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.

### Step 3

- Do not remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C.
Mix gently by swirling the microwell plate on a flat bench for 30 seconds. Cover the microwell plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.

**Mix gently**

Cover the microwell plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.

**Remove the Plate Sealer.**

Wash each well 5 times with 350 μL of Working Wash Buffer per well. Then remove the liquid. Turn the microwell plate upside down on absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried.

**Remove the Plate Sealer.**

Wash each well 5 times with 350 μL of Working Wash Buffer. Turn the microwell plate upside down on absorbent tissue.

**Mix gently**

Cover the microwell plate with the Plate Sealer and incubate at 37°C for 30 min.

**Add 100 μL of Conjugate to each well except for the Blank well. (Red Reagent)**

Then a blue color should develop in wells containing Positive specimens.

**Mix gently**

Cover the microwell plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.

**Add 50 μL of Stop Solution to each well. (Clear Reagent)**

Then a yellow color should develop in wells containing Positive specimens.

**Read at 450/630-700 nm in 30 minutes.**

Note: Microwell plate can also be read at 450 nm, but it is recommended to read it at 450/630 nm for better results.

**Interferences and Cross-Reactivity**

Interferences are not observed up to concentrations of 0.6 mg/mL Oxalic Acid, 0.1 mg/mL Ascorbic Acid, 0.1 mg/mL Caffeine, 0.6 mg/mL Oxalic Acid, 2 mg/mL Betalain, 2 mg/mL Hemoglobin, 1% Methanol and 1% Ethanol. Rheumatoid factors do not interfere with antibody binding. Cross-Reactivity are not observed in Syphilis, HBsAg, HIV, HCV, HSV1 IgG, Toxo IgG, and CMV IgG positive specimens.

**BIBLIOGRAPHY**


**Example of Calibrator 3 Calculation**

Example of Calibrator 3 Calculation

Interpretation of Results - Qualitative and Quantitative

### Intra-Assay

<table>
<thead>
<tr>
<th>Item Absorbance</th>
<th>Intra-Assay Cut-Off Value</th>
<th>Mean Absorbance of Calibrator 3 - Blank Absorbance</th>
<th>Cut-Off Value: Mean Absorbance of Calibrator 3 - Blank Absorbance</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.779</td>
<td>1.043</td>
<td>0.020</td>
<td>1.779 - 0.020 = 1.759</td>
<td>0.005</td>
<td>0.005</td>
<td>0.007</td>
<td>0.007</td>
</tr>
<tr>
<td>2.629</td>
<td>1.904</td>
<td>0.020</td>
<td>2.629 - 0.020 = 2.609</td>
<td>0.007</td>
<td>0.007</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>3.184</td>
<td>2.166</td>
<td>0.015</td>
<td>3.184 - 0.015 = 3.169</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Intra-Assay: Within-run precision has been determined by using 10 replicates of three specimens: a low positive, a medium positive and a high positive.

Inter-Assay: Between-run precision has been determined by 3 independent assays on the same three specimens: a low positive, a medium positive and a high positive. Three different lots of the Rubella IgG EIA Test Kit were used in testing these specimens over a period.

**LIMITATIONS**

1. The Rubella IgG EIA Test Kit is used for the detection of IgG antibodies to Rubella in human serum or plasma. Diagnosis of an infectious disease should not be established based on a single test result. Further testing, including confirmatory testing, should be performed before a specimen is considered positive. A negative test result does not exclude the possibility of exposure. Specimens containing precipitate may give inconsistent test results.

2. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.

3. As with other immune assays, there is the possibility that the positive result cannot be affected due to inadequate washing from the initial test. The results may be affected due to procedural or instrument error.

### PERFORMANCE CHARACTERISTICS

The calibrators are referenced to the World Health Organization International Standard for Anti-Rubella Serum (3rd International Standard preparation) at each concentration level.

### LIMIT OF DETECTION

The Rubella IgG EIA Test Kit has been certified by the World Health Organization to a mixed titer performance panel (PTR201, Boston Biomedica Inc). It has also been compared to a leading commercial Rubella IgG MEIA test using clinical specimens. The results of the rubella clinical sensitivity of the Rubella IgG EIA is 96.4%, and the clinical specificity is >99.9%.

**Interpretation of Results**

Calculate the Index Value by dividing the Specimen Absorbance by the Cut-Off Value, then read the result by referring to the Interpretation of Results table below.

**Note:** For equivocal results, the specimen should be retested. Specimens that are repeatedly equivocal after retesting should be confirmed using an alternate method. If the results remain equivocal, a new specimen in two technical replicates. If the new specimen is positive, the specimen is presumed to be positive.

**Definitions and Symbols**

- **For In vitro diagnostic use only**
- **For In vitro diagnostic use only**
- **Store between -20°C**
- **Store between -20°C**
- **Lot Number**
- **Substrate B**
- **Wash Buffer (2x)**
- **Calibrator 1**
- **Calibrator 3**
- **Plate Sealer**
- **Package Insert**
- **Microwell Plate**
- **Stop Solution**
- **Interface**
- **Intra-Assay**
- **Inter-Assay**
- **Reagents and Fixative**
- **Conjugate**
- **Calibrator 2**
- **Calibrator 4**

**BIBLIOGRAPHY**


**Acronyms**

- **ABSI:** Average Blank Subtraction Index
- **CVA:** Coefficient of Variation
- **EIA:** Enzyme Immunoassay
- **FCS:** Fractional Complement Saturation
- **HCMV:** Human Cytomegalovirus
- **HCV:** Hepatitis C Virus
- **HSV1:** Herpes Simplex Virus Type 1
- **HSV2:** Herpes Simplex Virus Type 2
- **IFN:** Interferon
- **Ig:** Immunoglobulin
- **IgG:** Immunoglobulin G
- **IgM:** Immunoglobulin M
- **IgA:** Immunoglobulin A
- **IMM:** Immunoreactivity
- **MEIA:** Microscopic Enzyme Immunoassay
- **MFI:** Mean Fluorescence Intensity
- **NFAT:** Nuclear Factor of Activated T cells
- **PBMC:** Peripheral Blood Mononuclear Cells
- **PCR:** Polymerase Chain Reaction
- **RA:** Rheumatoid Arthritis
- **SDS-PAGE:** Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
- **TNF:** Tumor Necrosis Factor
- **WHO:** World Health Organization

**Figure Legends**

- **Figure 1:** Western blot analysis of recombinant protein expression in E. coli
- **Figure 2:** ELISA analysis of recombinant protein binding
- **Figure 3:** Flow cytometry analysis of cell surface expression
- **Figure 4:** Immunofluorescence staining of target cells
- **Figure 5:** Electron microscopy of viral particles

**Tables**

- **Table 1:** Summary of experimental conditions
- **Table 2:** Results and statistical analysis
- **Table 3:** Comparative analysis of different methods

**Acknowledgments**

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**References**


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**Supplementary Information**

Additional data and resources are available in the online version of this article. These include supplementary methods, figures, and tables.

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