Rubella IgG EIA Test Kit
Catalog Number: 6208015

An enzyme immunoassay (EIA) for the qualitative and quantitative detection of IgG antibodies to Rubella in human serum or plasma.

For professional in vitro diagnostic use only.

SUMMARY

The Rubella IgG EIA Test Kit is an immunoassay for the qualitative and quantitative detection of the presence of IgG antibodies to Rubella in serum or plasma specimen. The test utilizes purified Rubella antigens to selectively detect IgG antibodies to Rubella in serum or plasma. Rubella is a small spherical enveloped RNA virus belonging to Togaviridae family. Most commonly known as the German or 3-day measles, the Rubella virus is spread through droplet infection resulting in mild contagious rash in children or 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 depends on the time the infection occurs and may result in fetal death, or congenital abnormalities. The Rubella IgG EIA Test Kit is an immunoassay for the quantitative detection of IgG antibodies to Rubella in serum or plasma. Rubella is a small spherical enveloped RNA virus belonging to Togaviridae family. Most commonly known as the German or 3-day measles, the Rubella virus is spread through droplet infection resulting in mild contagious rash in children or 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 depends on the time the infection occurs and may result in fetal death, or congenital abnormalities.

PRECAUTIONS

- For professional in vitro diagnostic use only. Do not use after expiration date.
- Follow the wash procedure to ensure optimum assay performance.
- Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.
- Use a new pipet tip for each specimen assayed.
- Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate. Do not allow wells to dry out during the assay procedure.
- 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.
- All equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer’s instructions.

HEALTH AND SAFETY INFORMATION

- Some components of this kit contain human blood derivatives. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.
- Wear disposable gloves and other protective clothing such as laboratory coats and eye protection while handling kit reagents and specimens. Wash hands thoroughly when finished.
- ProClin™ 300 is included as a preservative in the Conjugate, Concentrated Wash Buffer, Specimen Diluent, Substrate and Calibrators. Avoid any contact with skin or eyes.
- Do not eat, drink or smoke in the area where the specimens or kits are handled. Do not mouth pipette.
- Avoid any contact of Substrate A, Substrate B, and Stop Solution with skin or mucosa. The Stop Solution contains 2M sulfuric acid which is a strong acid. If spills occur, wipe immediately with large amounts of water. If the acid contacts the skin or eyes, flush with large amounts of water and seek medical attention.
- Non-disposable apparatus should be sterilized after use. The preferred method is to autoclave for one hour at 121°C. Disposables should be autoclaved or incinerated. Do not autoclave materials containing sodium hypochlorite.
- Handle and dispose all specimens and materials used to perform the test safely and properly.

The amount of Rubella IgG antibodies present in the specimens. Sulfuric acid solution is added to the microwell plate to stop the reaction producing a color change from blue to yellow. The color intensity, which corresponds to the amount of Rubella IgG antibodies present in the specimens, is measured with a microplate reader at 450/630-700 nm or 450 nm.
perform the test as if they contained infectious agents. Observe established precautions against microbiological hazards throughout all the procedures and follow the standard procedures for proper disposal of specimens.

- Observe Good Laboratory Practices when handling chemicals and potentially infectious material. Discard all contaminated material, specimens and reagents of human origin after proper decontamination and by following local, state and federal regulations.
- Neutralized acids and other liquids should be decontaminated by adding sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to a 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.

**STORAGE AND STABILITY**

- Unopened test kits should be stored at 2-8°C upon receipt. All reagents are stable through the expiration date printed on the box. Return reagents to 2-8°C immediately after use.
- Allow the sealed pouch to reach room temperature before opening the pouch and removing the required number of strips to prevent condensation of the microwell plate. The remaining unused strips should be stored in the original resealable pouch at 2-8°C and can be used within 1 month of the opening date.
- Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.

**SPECIMEN COLLECTION AND PREPARATION**

- The Rubella IgG EIA Test Kit can be performed using only human serum or plasma collected from venipuncture whole blood.
- EDTA, sodium heparin, and ACD collection tubes may be used to collect venipuncture whole blood and plasma specimens. The preservative sodium azide inactivates horseradish peroxidase and may lead to erroneous results.
- 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 matter may be capable of maintaining 37°C ± 2°C.
- Calibrated automatic or manual microwell plate washer capable of dispensing 350 µL/well.
- Freshly distilled or deionized water.
- Sodium hypochlorite solution for decontamination.
- Water bath or incubator capable of maintaining 37°C ± 2°C.
- **Materials required but not provided:**
  - Calibrated micropipettes capable of dispensing 5, 50 and 100 µL.
  - Vortex mixer for specimen mixing.
  - Timer.
  - Calibrated microplate reader capable of reading at 450 nm with a 630-700 nm reference filter, or reading at 450 nm without a reference filter.
  - Automated processor (optional).

**DIRECTIONS FOR USE**

Allow reagents and specimens to reach room temperature (15-30°C) prior to testing. The procedure must be strictly followed. Assay must proceed to completion within time limits. Arrange the controls so that well A1 is the Blank well. From well A1, arrange the controls in a horizontal or vertical configuration. The procedure below assigns specific wells arranged in a vertical configuration. Configuration may depend upon software.
Detailed Procedure

0. Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. Pour the contents of the bottle in a graduated cylinder and fill it with freshly distilled or deionized water to 1250 mL. It is stable for 2 weeks at 15-30°C. Note: If crystals are present in the Concentrated Wash Buffer, warm it up at 37°C until all crystals dissolve.

1. Add 100 μL of Calibrator 1 in wells B1 and C1. (Blue Reagent)
   - Add 100 μL of Calibrator 2 in wells D1 and E1. (Blue Reagent)
   - Add 100 μL of Calibrator 3 in wells F1 and G1. (Blue Reagent)
   - Add 100 μL of Calibrator 4 in wells H1 and A2. (Blue Reagent)
   - Leave A1 as Blank well.

2. Add 100 μL of Specimen Diluent to assigned wells starting at B2. The color of Specimen Diluent is green.
   - Add 5 μL of specimen to assigned wells starting at B2. A color change from green to blue will occur to verify that the specimen has been added.
   - Remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C.

3. Mix gently by swirling the microwell plate on a flat bench for 30 seconds.
   - Cover the microwell plate with the Plate Sealer and incubate at 37°C for 30 minutes ± 2 minutes.

4. Remove the Plate Sealer.
   - Wash each well 5 times with 350 μL of Working Wash Buffer, then remove the liquid. Turn the microwell plate upside down on an absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried. Note: Improper washing may cause false positive results.

5. Add 100 μL of Conjugate to each well except for the Blank well. The color of Conjugate is red.
   - Remove and store unused strips at 2-8°C.

6. 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.

7. Repeat Step 4.
   - Repeat Step 4

8. Add 50 μL of Substrate A to each well. (Clear Reagent)
   - Add 50 μL of Substrate B to each well. (Clear Reagent) Then a yellow color should develop in wells containing Positive specimens.

9. Mix gently then cover microwell plate with Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 10 minutes ± 1 minute.
   - Mix then cover microwell plate with Plate Sealer and incubate at 37°C for 10 min

10. Remove the Plate Sealer.
    - Add 50 μL of Stop Solution to each well. (Clear Reagent) Then a blue color should develop in wells containing Positive specimens.

11. 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 450/630-700 nm for better results.

Simplified Procedure

0. Leave A1 as Blank well.

1. Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25.

2. Starting B2: Add 100 μL Specimen Diluent
   - Starting B2: Add 5 μL specimen
   - Remove and store unused strips at 2-8°C

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

4. Remove the Plate Sealer.
   - Wash each well 5 times with 350 μL of Working Wash Buffer, then remove the liquid. Turn the microwell plate upside down on an absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried. Note: Improper washing may cause false positive results.

5. Add 100 μL of Conjugate to each well except for the Blank well.
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6. Cover the microwell plate with the Plate Sealer and incubate at 37°C ± 2°C for 30 minutes ± 2 minutes.

7. Repeat Step 4.
   - Repeat Step 4

8. Add 50 μL of Substrate A to each well. (Clear Reagent)
   - Add 50 μL of Substrate B to each well. (Clear Reagent) Then a yellow color should develop in wells containing Positive specimens.

9. Mix gently then cover microwell plate with Plate Sealer and incubate at 37°C ± 2°C for 10 minutes ± 1 minute.

10. Remove the Plate Sealer.
    - Add 50 μL of Stop Solution to each well. (Clear Reagent) Then a blue color should develop in wells containing Positive specimens.

11. 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 450/630-700 nm for better results.

**INTERPRETATION OF RESULTS**

**Qualitative**

Calculate the Index Value to obtain qualitative specimen results.

1. If the test is valid, obtain Cut-Off Value by subtracting the Blank Absorbance from the Mean Absorbance of Calibrator 3. See an example of Cut-Off Value calculation below.

<table>
<thead>
<tr>
<th>Item</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank Absorbance: Well A1</td>
<td>0.014</td>
</tr>
<tr>
<td>Cut-Off Value: Mean Absorb of Calibrator 3 – Blank Absorb.</td>
<td>1.057 – 0.014 = 1.043</td>
</tr>
</tbody>
</table>

**CALCULATION OF RESULTS AND VALIDITY**

1. Calculate the Mean Absorbance of Calibrators 1-4 by referring to the table below.

**Example of Calibrator 3 Calculation**

<table>
<thead>
<tr>
<th>Item</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator 3: Well F1</td>
<td>1.012</td>
</tr>
<tr>
<td>Calibrator 3: Well G1</td>
<td>1.102</td>
</tr>
<tr>
<td>Total Absorbance of Calibrator 3</td>
<td>1.012 + 1.102 = 2.114</td>
</tr>
<tr>
<td>Mean Absorbance of Calibrator 3</td>
<td>2.114 / 2 = 1.057</td>
</tr>
</tbody>
</table>

2. Check the validation requirements below to determine if the test results are valid.

<table>
<thead>
<tr>
<th>Item</th>
<th>Validation Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank Well</td>
<td>Blank Absorbance should be &lt; 0.050 if read at 450/630-700 nm Note: It should be &lt; 0.100 if read at 450 nm</td>
</tr>
<tr>
<td>Calibrator 1</td>
<td>Mean Absorbance &gt; 0.200 and &lt; 0.700 after subtraction of Blank Absorbance</td>
</tr>
<tr>
<td>Calibrator 2</td>
<td>Mean Absorbance &gt; 0.200 and &lt; 0.700 after subtraction of Blank Absorbance</td>
</tr>
<tr>
<td>Calibrator 3</td>
<td>Mean Absorbance &gt; Calibrator 2 and &lt; Calibrator 4 after subtraction of Blank Absorbance</td>
</tr>
<tr>
<td>Calibrator 4</td>
<td>Mean Absorbance &gt; 1.500 after subtraction of Blank Absorbance</td>
</tr>
</tbody>
</table>

**NOTE:** The test results are considered invalid if the above validation requirements are not met. Repeat the test or contact your local distributor.

**AUTOMATED PROCESSING**

Automatic EIA microplate processors may be used to perform the assay after validating the results to ensure they are equivalent to those obtained using the manual method for the same specimens. Incubation times may vary depending on the processors used but do not program less incubation times than the procedure listed above. When automatic EIA microplate processors are used, periodic validation is recommended to ensure proper results.

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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 sensitive immunoassays, there is the possibility that the positive result cannot be repeated due to inadequate washing from the initial test. The results may be affected due to procedural or instrument error.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The Rubella IgG EIA Test Kit has correctly identified specimens of a mixed titer performance panel and has been compared to a leading commercial Rubella IgG EIA test using clinical specimens. The results show that the clinical sensitivity of the Rubella IgG EIA Test Kit is 96.4%, and the clinical specificity is >99.9%.

Rubella IgG EIA vs. Other EIA

<table>
<thead>
<tr>
<th>Method</th>
<th>Other EIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>54</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Total Results</td>
<td>54</td>
</tr>
</tbody>
</table>

Clinical Sensitivity: 96.4% (87.7-99.6%)*
Clinical Specificity: >99.0% (90.5-100.0%)*
Overall Agreement: 97.9% (92.4-99.7%)*
*95% Confidence Interval

Reproducibility

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

Inter-Assay: Between-run precision has been determined by 3 independent assays on the same two specimens: a low positive and a medium positive. Three different lots of the Rubella IgG EIA Test Kit have been tested using these specimens over a 5-day period.
### Rubella IgG EIA Test Kit

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**QUALITY SYSTEM CERTIFIED**

ISO 9001  ISO 13485

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### intra-assay and inter-assay variability

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Intra-Assay</th>
<th>Inter-Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Absorb./ Cut-Off</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>1</td>
<td>1.206</td>
<td>0.065</td>
</tr>
<tr>
<td>2</td>
<td>1.884</td>
<td>0.111</td>
</tr>
</tbody>
</table>

### REFERENCES