The Trinity Biotech Capita™ Rubella IgM Enzyme-Linked Immunosorbent Assay (ELISA) is intended for the in vitro qualitative determination of IgM antibodies in human serum and as an aid in the diagnosis of current or recent infection with Rubella virus. High complexity test.

INTENDED USE
The Trinity Biotech Capita™ Rubella IgM Enzyme-Linked Immunosorbent Assay (ELISA) is intended for the in vitro qualitative determination of IgM antibodies in human serum and as an aid in the diagnosis of current or recent infection with Rubella virus.

INTRODUCTION
Rubella virus infection, also known as three-day or “German measles”, is a viral exanthematosous infectious disease typically subclinical or mild disease in children and young adults. Symptomatology is generally characterized by fever, maculopapular rash accompanied by enlargement of lymph nodes. Infection during pregnancy, particularly the first trimester, can result in fetal death or severe anatomical deformity and mental retardation. Therefore, early detection of Rubella infection is extremely important. In a patient suffering from a primary rubella infection, the appearance of both IgG and IgM antibodies is associated with the appearance of clinical signs and symptoms when present.

IgM antibodies become detectable in a few days after the onset of signs and symptoms and reach peak levels at 7 to 10 days. These antibodies persist but rapidly diminish in concentration over the next 4 to 5 weeks until the antibody is no longer clinically detectable. Production of IgG increases rapidly for the next 7 to 21 days, then level off or even decrease in strength. IgG antibodies remain present and protective indefinitely.

Peak levels at 7 to 10 days. These antibodies persist but rapidly diminish in concentration over the first month, so during the first three to five months of life the total IgG level falls. After this time, the postnatal rubella, may continue to produce rubella-specific IgM for several months, and during this period it may become the dominant antibody. The half-life of passively transferred IgG is about one month, so during the first three to five months of life the total IgG level falls. After this time, the infant begins to produce its own IgG and the level again rises.

Voller and Biedwetz, using a crude viral antigen, were the first ones to apply ELISA in rubella serology. The identification of IgM antibodies by enzyme immunoassay has resulted in potential sources of error caused by IgG and IgM rheumatoid factor interferences. Because immune IgG is involved in both false-positive and false-negative IgM reactions, most methods for avoiding incorrect results concentrate on treating the patient’s sample to remove IgG. This test uses the ELISA technology utilizing high purity antigen and serum treatment solution to remove the interferences potentially caused by IgG and IgM-RF.

The sensitivity, specificity, and reproducibility of Enzyme-Linked Immunosorbent Assays are comparable to other serological tests for antibody, such as immunofluorescence, complement fixation, hemaggglutination and radioimmunoassay.

PRINCIPLE OF THE ASSAY
Enzyme-Linked Immunosorbent Assays (ELISA) rely on the ability of biological materials (e.g., antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid-phase are in direct contact with a patient’s serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen-antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgM globulin conjugated with horseradish peroxidase which will bind to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of Chromogen/Substrate tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient’s serum, a blue color develops. When the enzymatic reaction is stopped with 1N H2SO4, the contents of the wells turn yellow. The color, which is indicative of the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microtiter plate reader.

STORAGE AND STABILITY
1. Store unopened kit between 2° and 8° C. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Unopened microassay plates must be stored between 2° and 8° C. Unused strips must be immediately resealed in a sealable bag with desiccant and returned to storage between 2° and 8° C.
3. Store HRP Conjugate between 2° and 8° C.
4. Store the Calibrator, Positive and Negative Controls between 2° and 8° C.
5. Store Serum Diluent Plus and 2X Wash Buffer Solution between 2° and 8° C.
6. Store the Chromogen/Substrate Solution Type I between 2° and 8° C. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells.
7. Store 1X (diluted) Wash Buffer Type I at room temperature (21° to 25° C) for up to 5 days, or up to 1 week between 2° and 8° C.

Note: If constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit. Refer to package label for expiration date. Precautions were taken in the manufacture of this product to protect the reagents from contamination and bacteriostatic agents have been added to the liquid reagents. Care should be exercised to protect the reagents in this kit from contamination. Do not use if evidence of microbial contamination or precipitation is present.

MATERIALS SUPPLIED
Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label.

1. Purified Rubella antigen coated microassay plate: 96 wells, configured in twelve 8x strips stored in a foil pouch with desiccant. Allow the wells to equilibrate to room temperature (21°-25°C) in the pouch to protect from condensation. When stored at 2°-8°C, coated strips are stable until the labeled expiration date. (96T: one plate, 480T: five plates)
2. Calibrator: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Calibrator is used to calibrate the assay to account for day-to-day fluctuations in temperature and other testing conditions. (96T: one vial, 0.4 mL, 480T: one vial, 0.8 mL) *
3. Positive Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Positive Control is utilized to control the positive range of the assay. (96T: one vial, 0.4 mL, 480T: one vial, 0.8 mL) *
4. Negative Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Negative Control is utilized to control the negative range of the assay. (96T: one vial, 0.4 mL, 480T: one vial, 0.8 mL) *
5. Horseradish peroxidase (HRP) Conjugate: Ready to use. Goat anti-human IgM containing ProClin® (0.1%) and gentamicin as preservatives. (96T: one bottle, 16 mL, 480T: five bottles, 16 mL)
6. Serum Diluent Plus: Ready for use. Contains goat/sheep anti-human IgG for serum absorption to remove competing IgG, with protein stabilizers and ProClin® (0.1%) as a preservative. (96T: two bottles, 45 mL each, 480T: two bottles, 0.225 mL each)
7. Wash Buffer Type I (20X concentrate): dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween-20 and ProClin® (0.1%) as a preservative. (96T: one bottle, 50 mL, 480T: one bottle, 250 mL)
8. Chromogen/Substrate Solution Type I: Tetramethylbenzidine (TMB), ready to use. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells. (96T: one bottle, 15 mL, 480T: five bottles, 15 mL)
9. Stop Solution: Ready to use, contains a 1N H2SO4 solution. (96T: one bottle, 15 mL, 480T: five bottles, 15 mL)

* Note: serum vials may contain excess volume.

The following components are not kit lot dependent and may be used interchangeably within the Trinity Biotech ELISA IgM assays: Chromogen/Substrate Solution Type I, Wash Buffer Type I, and Stop Solution. Please check that the appropriate Trinity Biotech Reagent Type (I, II, etc.) is used for the assay.

ADDITIONAL REQUIREMENTS
- Wash bottle, automated or semi-automated microwell plate washing system.  
- Microtubes, including multichannel, capable of accurately delivering 10-200 μL volumes (less than 3% CV)  
- One liter graduated cylinder.
- Paper towels.
- Test tube for serum dilution.
- Reagent reservoirs for multichannel pipettes.
- Pipette tips.
- Distilled or deionized water (ddH2O), CAP (College of American Pathology) Type 1 or 2 or equivalent.
- Timer capable of measuring to an accuracy of +/- 1 second (0 – 60 minutes).
- Disposable basins and 0.5% sodium hypochlorite (50 mL bleach in 950 mL ddH2O).
- Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, the sensitivity filter to 600-650 nm. Read the Operator’s Manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.

Note: Use only clean, dry glassware.

PRECAUTIONS
1. For in vitro diagnostic use.
2. The serum component used in the preparation of the Controls and Calibrator in this kit have been tested by an FDA approved method for the presence of antibodies to human immunodeficiency virus (HIV 1 & 2; HIV 183), hepatitis B (HCV) as well as hepatitis B surface antigen and found negative. Because no test method can offer complete assurance that HIV, HCV, hepatitis B virus, or other infectious agents are absent, specimens and human-based reagents should be handled as if they do not contain infectious agents.
3. The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.
4. The components in this kit have been quality control tested as a Master Lot unit. Do not mix components from different lot numbers except Chromogen/Substrate Solution Type I, Stop Solution, Wash Buffer Type I. Do not mix with components from other manufacturers.
5. Do not use reagents beyond the stated expiration date marked on the package label.
6. All reagents must be at room temperature (21° to 25°C) before running assay. Remove only the volume of reagents that is needed. Do not pour reagents back into vials as reagent contamination may occur.
7. Before opening Control and Calibrator vials, tap firmly on the bench top to ensure that all liquid is at the bottom of the vial.
8. Use only distilled or deionized water and clean glassware.
9. Do not let reagents dry out before use; add reagents immediately after completing wash steps.
10. Avoid cross-contamination of reagents. Wash hands before and after handling reagents.
11. Cross-contamination of reagents and/or samples could cause false results.
12. If washing steps are performed manually, wash all wells 3 times. If using automated equipment, wash cycles may be necessary if a washing manifold or automated equipment is used.
13. The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.
14. Never pipeette by mouth or allow reagents or patient sample to come into contact with skin. Reagents containing ProClin® 200, sodium azide, and TMB may be irritating. Avoid contact with skin and eyes. In case of contact, flush with plenty of water.

15. If a sodium hypochlorite (bleach) solution is being used as a disinfectant, do not expose to work area during actual test procedure because of potential interference with enzyme activity.

16. Avoid contact of Stop Solution (1N sulfuric acid) with skin or eyes. If contact occurs, immediately flush area with water.

17. Caution: Liquid waste at acid pH must be neutralized prior to adding sodium hypochlorite (bleach) solution to avoid formation of poisonous gas. Recommend disposing of reacted, stopped plates in biohazard bags. See Precaution 3.

18. The concentrations of anti-Rubella in a given specimen determined with assay kits from different manufacturers can vary due to differences in assay methods and reagent specificity.

The safety data sheet is available upon request.

**WARNING**

Serum Diluent, Conjugate, and Wash Buffer contain 0.1% ProClin® 200®, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

H317: May cause an allergic skin reaction.

**PREPARATION FOR THE ASSAY**

1. All reagents must be removed from refrigeration and allowed to come to room temperature before use (21° to 25° C). Return all reagents to refrigerator promptly after use.

2. All samples and controls should be vortexed before use.

3. Dilute 50 mL of the 20X Wash Buffer Type I to 1 L with distilled and/or deionized H2O. Mix well.

**SERUM TREATMENT**

Solid phase immunassays for the detection of virus-specific IgM are known to be sensitive to interfering factors. The goal is to use an IgM preparation in the Serum Diluent Plus Solution that diminishes competing virus-specific IgM, which would be responsible for false negative reactions. False positives are similarly minimized by removing the IgM, thus neutralizing the bound rheumatoid factor in the samples.

**ASSAY PROCEDURE**

1. Place the desired number of strips into a microwell frame. Allow four (4) Control/Calibrator determinations (one Negative Control, two Calibrators, and one Positive Control) per run. A reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Control/Cutoff Calibrator configurations. Run unused strips to the sealable bag with desiccant, seal, and immediately refrigerate.

2. Dilute test sera, Calibrator and Control sera 1:1 (e.g., 10 mL + 800 mL) in Serum Diluent Plus. (For manual dilutions it is suggested to dispense the Serum Diluent into the test tube first and then add the patient serum). Mix well (Vortexing recommended).

3. To individual wells add 100 µL of diluted patient sera, Calibrator and Control sera. Add 100 µL of Serum Diluent Plus to the reagent blank well. Check software and reader requirements of the correct reagent blank well configuration.

4. Incubate each well at room temperature (21° to 25° C) for 15 minutes ± 2 minutes. Incubate each well 30 minutes ± 2 minutes at room temperature (21° to 25° C). Repeat wash as described in Step 5.

5. Add 100 µL of Chromogen/Substrate solution (TMB) to each well, reagent blank well, maintaining a constant rate of addition across the plate. Incubate each well at room temperature (21° to 25° C) for 15 minutes ± 2 minutes.

6. Stop reaction by addition of 100 µL of Stop Solution (1N H2SO4) following the same order of Chromogen/Substrate addition, including reagent blank well. Tap the plate gently along the outsides to mix contents of the wells. The plate may be held up to one (1) hour after addition of the Stop Solution before reading.

12. The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. If dual wavelength is used, set the reference filter to 600-650. The instrument should be calibrated on the outside of the plate. The plate may be held up to one (1) hour after addition of the Stop Solution before reading.

**METHODS FOR USE**

**SPECIMEN COLLECTION AND STORAGE**

1. Handle all blood and serum as if capable of transmitting infectious agents.

2. Optimal performance of the kit depends upon the use of fresh serum samples (clear, non-hemolyzed, non-icteric, non-clot). A minimum volume of 50 µL is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture. Early separation from the clot prevents hemolysis of serum.

3. Store serum between 2° and 8° C if testing will take place within two days. If specimens are to be kept for longer periods, store at -20° C or colder. Do not use a frost-free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield erroneous results.


**INTERPRETATION**

1. The concentrations of anti-Rubella in a given specimen determined with assay kits from different manufacturers can vary due to differences in assay methods and reagent specificity.

**QUALITY CONTROL**

For the assay to be considered valid the following conditions must be met:

1. Calibrator and Controls must be run with each test run.

2. Reagent blank (when read against air blank) must be <0.150 Absorbance (A) at 450 nm.

3. Negative Control must be ≤ 0.250 at 450 nm (when read against reagent blank).

4. Each Calibrator must be ≥ 0.300 at 450 nm (when read against reagent blank).

5. Cutoff Calibrator Value must be ≥ 0.250 at 450 nm (when read against reagent blank).

6. The ISR for the Positive and Negative Controls should be in their respective ranges printed on the vials. If the Control values are not within their respective ranges, the test should be considered invalid and the test should be repeated.

**CALCULATIONS**

1. Mean Calibrator O.D. (Optical Density) - Calculate the mean O.D. value for the Calibrator from the two Calibrator determinations.

2. Correction Factor - To account for day-to-day fluctuations in assay activity due to room temperature and timing, a Correction Factor is determined by Trinity Biotech for each lot of kits. The Correction Factor is printed on the Calibrator vial.

3. Cutoff Calibrator Value = The Cutoff Calibrator Value for each assay is determined by multiplying the Correction Factor by the mean Calibrator O.D. determined in Step 1.

4. ISR Value - Calculate an Immune Status Ratio (ISR) for each specimen by dividing the specimen O.D. Value by the Cutoff Calibrator Value determined in Step 3. 

**ANALYSIS**

1. The patients’ ISR (Immune Status Ratio) values are interpreted as follows:

<table>
<thead>
<tr>
<th>ISR Value</th>
<th>Interpretation</th>
<th>Equivalents</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.90</td>
<td>Negative No significant level of detectable Rubella IgM antibody.</td>
<td>≤ 0.90</td>
<td>0.91-1.0</td>
</tr>
<tr>
<td>≥ 1.10</td>
<td>Significant level of detectable Rubella IgM antibody. Indicative of current or recent infection.</td>
<td>≥ 1.01</td>
<td>&gt; 1.10</td>
</tr>
</tbody>
</table>

2. Samples that remain equivocal after repeat testing should be tested on an alternate method, e.g., immunofluorescence assay (IFA).
A total of 200 random serum samples collected from US blood centers; 100 from blood centers in California and 100 from blood centers on the east coast were tested to establish the expected Rubella infection. Table 1 summarizes the distribution of Trinity Biotech Rubella IgM (Catalog #2325360) assay ISR Values observed for the population.

Table 1: Distribution of Trinity Biotech Rubella IgM (Catalog #2325360) Assay ISR Values from 200 US Individuals

<table>
<thead>
<tr>
<th>Rubella IgM Distribution of ISR Values in a Normal Population (n=200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
</tr>
<tr>
<td>0.00-0.20</td>
</tr>
<tr>
<td>0.21-0.40</td>
</tr>
<tr>
<td>0.41-0.60</td>
</tr>
<tr>
<td>0.61-0.80</td>
</tr>
<tr>
<td>0.81-1.00</td>
</tr>
<tr>
<td>1.11-1.20</td>
</tr>
<tr>
<td>1.21-1.40</td>
</tr>
<tr>
<td>1.41-1.60</td>
</tr>
<tr>
<td>1.61-1.80</td>
</tr>
<tr>
<td>1.81-2.00</td>
</tr>
</tbody>
</table>

LIMITATIONS OF USE

1. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.

2. The results of ELISA immunoassays performed on serum from immunosuppressed patients must be interpreted with caution.

3. This device is not intended for the determination of immune status. It is intended for the determination of a person’s immune response to indicate primary infection, reinfection or virus reactivation to Rubella virus.

4. Samples that remain equivocal after repeat testing should be retested by an alternate method, e.g., immunofluorescence assay (IFA). If results remain equivocal upon further testing, an additional sample should be taken.

5. The absence of detectable IgM antibody does not rule out the possibility of recent or current infection. However, if Rubella infection is still suspected, obtain a second specimen 5-7 days later and repeat the testing.

6. As is the case with other serologic techniques, the Trinity Biotech Rubella IgM test does not differentiate between vaccine and active virus infection antibodies.

7. Serum obtained after the signs of a rash may be declining in IgM response, therefore, the test may read negative. It is recommended that neonate’s and mother’s serum samples be tested in parallel. The presence of IgM antibody in the neonate’s serum can be considered indicative of congenital infection only if there has not been placental leakage. Additionally, if the infant has a congenital infection, the IgM antibody (and IgG antibody) level may persist or rise, whereas if the source of the antibody is maternal, the neonate’s antibody level will drop in parallel to the half-life of that immunoglobulin.

14. All positive test results require careful interpretation in combination with other serologic and clinical observations. Positive results should be confirmed by an alternate method.

REFERENCES


**CAPTIA™ RUBELLA IGM SUMMARY OF ASSAY PROCEDURE**

1. DILUTE SERUM 1:81
2. ADD TO MICROWELLS 100µl
3. WASH
4. ADD CONJUGATE 100µl
5. WASH
6. ADD TM8 SOLUTION 100µl
7. ADD STOP 100µl tris-HCl
8. READ AT 450NM

The safety data sheet is available upon request.

**WARNING**
Serum Diluent, Conjugate, and Wash Buffer contain 0.1% ProClin 3000®, a biocidal preservative that may cause sensitization by skin contact, prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

H317: May cause an allergic skin reaction.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352: IF ON SKIN: Wash with plenty of soap and water.
P333 + P313: If skin irritation or rash occurs: Get medical advice/attention.
P501: Dispose of contents/container in accordance to local, regional, national and international regulations.

**WARNING**
Serum Diluent and Controls contain < 0.1% sodium azide.

H302: Harmful if swallowed.
P264: Wash thoroughly with plenty of soap and water after handling.
P270: Do not eat, drink or smoke when using this product.
P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P330: If swallowed, rinse mouth.
P501: Dispose of contents/container in accordance to local, regional, national and international regulations.

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**ORDERING INFORMATION**

<table>
<thead>
<tr>
<th>Catalog No.</th>
<th>Item</th>
<th>Quantity</th>
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</thead>
<tbody>
<tr>
<td>2325360</td>
<td>CAPTIA™ Rubella Igm Test Kit</td>
<td>96 Tests</td>
</tr>
<tr>
<td>2325361</td>
<td>CAPTIA™ Rubella Igm Test Kit</td>
<td>480 Tests</td>
</tr>
</tbody>
</table>

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**CONTROL**
- High Pos or Positive Control
- Low Pos or Cut-Off Control
- Negative Control

**EC REP**
- Authorized Representative

**CAUTION**
- Consult accompanying documents
- Wear protective gloves / protective clothing / eye protection / face protection.

**REFERENCE**
- Product Number
- Calibrator
- Lot
- Coefficient Factor
- Range
- Standard
- For In Vitro Diagnostic use

**STORAGE**
- Store at 2-8°C
- Store at 2-30°C

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