# Rubella IgM

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## IgM antibodies to Rubella virus (ELISA)

**REF: DS180604** Σ **96**

**Intended use**
The DiaSino Rubella IgM assay is an enzyme-linked immunosorbent assay (ELISA) for the in vitro qualitative determination of IgM antibodies to rubella virus in Human serum and plasma.

**Summary**[1-7]
Rubella, also known as German measles or three-day measles, is an infection caused by the rubella virus. This disease is often mild with half of people not realizing that they are infected. A rash may start around two weeks after exposure and last for three days. It usually starts on the face and spreads to the rest of the body.
Rubella is a common infection in many areas of the world. Each year about 100,000 cases of congenital rubella syndrome occur. Rates of disease have decreased in many areas as a result of vaccination. There are ongoing efforts to eliminate the disease globally.

**Test principle**
Capture method. Total duration of assay: **70 minutes**.
This kit uses capture ELISA principle to detect RV IgM. Polystyrene microwell strips are pre-coated with mouse anti-human IgM (anti-μ chain specific). During the first incubation step, all IgM antibodies binds to mouse anti-human IgM monoclonal antibody coated in microplate including the Anti-RV IgM, the wells are washed to remove unbound serum proteins, and Rubella antigens conjugated to the enzyme horseradish peroxidase (HRP-Conjugate) are added. During the second incubation step, if there is RV IgM antibody in sample, HRP-conjugated antigens will be bound to the anti-human RV IgM complexes previously formed and the unbound HRP-conjugate is then removed by washing. Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance.
The amount of color intensity can be measured and it is proportional to the amount of antibody captured in the wells, and to the amount of antibody in the sample respectively.

**Reagents**
**Materials provided**
- **Coated Microplate - symbol** RV IgM PLATE 8 x 12 strips, 96 wells.
- **Enzyme Conjugate - symbol** RV IgM CONJ 1 vial, 11.0 mL of HRP (horseradish peroxidase) labeled with Rubella antigen. Contains 0.1% ProClin300 preseRV IgMative.
- **Sample Diluent - symbol** RV IgM DILUT 1 vial, 11 mL, ready to use
- **Positive Control - symbol** RV IgM PC 1 vial, 0.2 mL.
- **Negative Control - symbol** RV IgM NC 1 vial, 0.2 mL
- **Substrate - symbol** SUBSTRATE 1 vial, 11mL, ready to use, (tetramethylbenzidine) TMB.
- **Stop Solution - symbol** STOP 1 vial, 6.0 mL of 1 mol/L sulfuric acid.
- **Wash Solution Concentrate - symbol** WASH 40X 1 vial, 25 mL (40X concentrated), PBS-Tween wash solution.
- **IFU -** 1 copy.
- **Plate Lid**: 2 pieces.

**Materials required (but not provided)**
- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 50 μL with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

**Precautions and warnings**
- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- The Stop Solution contains sulfuric acid, which can cause severe burns. In the event of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05% or 0.1% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- Substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

**Storage and stability**
- Store at 2-8°C.
- Seal and return unused reagents to 2-8°C, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

**Specimen collection and preparation**
- Human serum or plasma is recommended for this assay.
- Cap and store the samples at 18-25 °C for no more than 8 hours. Stable for 7 days at 2-8°C, and 1 month at -20°C. Freeze only once.
- Do not use heat-inactivated samples.
- Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation.
- Avoid grossly hemolytic, lipemic or turbid samples.

**Wash solution (40X dilution)**
Add deionized water to the 40X concentrated Wash Solution Concentrate. Dilute 25 mL of Wash Solution Concentrate with 975 mL of deionized water to a final volume of 1000 mL. Stable for 2 months at room temperature.

**Test procedure**
Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement. Mix all reagents through gently inverting prior to use.
- **Preparation**: Mark two wells as Negative control (e.g. B1, C1), two wells as Positive control (e.g. D1, E1) and one Blank (e.g. A1, neither samples nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.
- **Adding Sample Diluent:** Add **100 μL** of Sample Diluent into their respective wells except the Blank.
- **Adding Sample**: Add **10 μL** of **Positive control, Negative control,** and **Specimen** into their respective wells except the Blank. Mix by tapping the plate gently. Use a separate disposal pipette tip for each specimen to avoid cross-contamination.
  **Note:** After adding Sample, the reagents in wells turns **Blue** color from **Green.**
- **Incubating:** Cover the plate with the plate cover and incubate for **30 minutes at 37°C**.
- **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Wash Buffer. Each time allow the microwells to soak for **30-60 seconds**. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
- **Adding Conjugate:** Add **100 μL** of **Conjugate** into each well except the Blank.
- **Incubating:** Cover the plate with the plate cover and incubate for **30 minutes at 37°C**.
- **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Wash Buffer. Each time allow the microwells to soak for **30-60 seconds**. After the final washing cycle, turn down

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the plate onto blotting paper or clean towel and tap it to remove any remainders.
- **Coloring:** Add **100 µL** of **Substrate** into each well including the Blank. Incubate the plate at room temperature for 10 minutes avoiding light. The enzymatic reaction between the TMB substrate and the HRP-Conjugate produces blue color in Positive control and in positive sample wells.
- **Stopping:** Using a multichannel pipette or manually, add **50 µL** of **Stop Solution** into each well and mix gently. Intensive yellow color develops in Positive control and RV IgM positive sample wells.
- **Measuring:** Calibrate the plate reader with the Blank well and read the absorbance at **450nm**. If a dual filter instrument is used, set the reference wavelength at **630nm**.

Calculate the Cut-off value and evaluate the results. (**Note:** read the absorbance within **10 minutes** after stopping the reaction).

**Quality control and calculation**
- Read the sample's optical density (OD) at 450nm with a micro plate reader.
- Mean negative control OD value ≤ 0.1 and positive control OD value ≥ 0.8, the test is valid, otherwise the test is invalid.
- Cut-Off value (C.O.) = Mean negative control OD value + 0.10 (Calculated by 0.05 when Mean negative control OD value is ≤ 0.05, calculated by actual value when Mean negative control OD value is > 0.05).

**Positive Results: Sample O.D value ≥ C.O.**
Specimens giving an absorbance equal to or greater than the Cut-Off value are considered initially reactive, which indicates that RV IgM has probably been detected using RV IgM ELISA. All initially reactive specimens should be retested in duplicates using RV IgM ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for RV IgM with RV IgM ELISA.

**Negative Results: Sample OD value < C.O**
Specimens giving absorbance less than the Cut-Off value are negative for this assay, which indicates that no RV IgM has been detected with RV IgM ELISA, therefore the patient is probably not infected with Rubella and the blood does not contain RV IgM.

**Limitations**
- Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
- Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.

**Performance Characteristics**
**Specificity**
A study of 196 individuals was tested with DiaSino ELISA kits and found that the specificity was 99.25%.

**Sensitivity**
Among 232 RV IgM confirmed were positive when tested with this ELISA, and 231 samples of which were detected with positive, the sensitivity was 99.57%.

**Precision**
**Intra-assay**
CV ≤ 15%
Within-run precision has been determined by using 15 replicates of three specimens: a low positive, medium positive and a high positive.

**Inter-assay**
CV ≤ 20%
Between-run precision has been determined by 3 independent assays on the same three specimens: a low positive, medium positive and a high positive. Three different lots of the Rubella IgM ELISA Test Kit have been tested using these specimens over a 5-day period.

**Analytical specificity**
- 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 Bilirubin, 2 mg/mL Hemoglobin, 1 % Methanol and 1% Ethanol .
- Rheumatoid factors do not interfere with test .
- Cross-Reactivity are not observed in Syphilis, HBsAg ,HIV,HCV ,HSV1 IgM ,Toxo IgM and CMV IgM positive specimens.

**Reference**

1. Atkinson, William (2011). Epidemiology and Prevention of Vaccine-Preventable Diseases (12 ed.). Public Health Foundation. pp. 301–323. ISBN 9780983263135. Archived from the original on 2017-05-01. Retrieved 2017-05-05.
2. Huong McLean (2014). "3 Infectious Diseases Related To Travel". CDC health information for international travel 2014 : the yellow book. ISBN 9780199948499. Archived from the original on 2015-04-24.
3. Lambert, N; Strebel, P; Orenstein, W; Icenogle, J; Poland, GA (7 January 2015). "Rubella". Lancet. 385: 2297–307. doi:10.1016/S0140-6736(14)60539-0. PMC 4514442. PMID 25576992.
4. Neighbors, M; Tannehill-Jones, R (2010). "Childhood diseases and disorders". Human diseases (3rd ed.). Clifton Park, New York: Delmar, Cengage Learning. pp. 457–79. ISBN 978-1-4354-2751-8.
5. "Rubella vaccines: WHO position paper". Wkly Epidemiol Rec. 86 (29): 301–16. 15 July 2011. PMID 21766537. Archived from the original on 5 June 2015.
6. Best JM (2007). "Rubella". Semin Fetal Neonatal Med. 12 (3): 182–92. doi:10.1016/j.siny.2007.01.017. PMID 17337363.
7. Stegmann BJ, Carey JC (2002). "TORCH Infections. Toxoplasmosis, Other (syphilis, varicella-zoster, paRV IgMovirus B19), Rubella, Cytomegalovirus (CMV), and Herpes infections". Curr Women's Health Rep. 2 (4): 253–8. PMID 12150751.


**Symbols**

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|---|---|---|---|
| IVD<br>**In vitro diagnostic medical device** | 8°C 2°C<br>**Temperature limit** | **Consult instructions for use** | REF<br>**Catalog number** |
| LOT<br>**Batch code** | **Date of manufacture** | **Use-by date** | Σ<br>**Contains sufficient for <n> tests** |
| **Manufacturer** | **Do not use if package is damaged and consult instructions for use** | | |


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