

## Rubella IgM Elisa

Catalog No. E-RVM-K15



### μ-capture IgM ELISA



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### INTENDED USE

The RD-RatioDiagnostics E-RVM-K15 Rubella IgM u-capture ELISA test system is an Enzyme-Linked Immunosorbent Assay kit providing material for the detection of IgM-class antibodies to Rubella virus in human serum or plasma in order to confirm the primary Rubella infection. This assay is intended for *in vitro* use only.

### SUMMARY AND EXPLANATION

The Rubella virus, most commonly known as the German or 3-day measles is an RNA virus that belongs to the family Togaviridae and contains three major structural proteins, E1, E2 and C. It is spread through direct or droplet contact from nasopharyngeal secretions. The infection is highly contagious and affects children, 5-14 years as well as young adults. Rubella infection, however, is largely benign with symptoms ranging from subclinical to a disease characterized by an erythematous rash, low-grade fever, headache, lymphadenopathy, arthralgia, and conjunctivitis. Immunizations and natural infection both confer lifelong immunity and reinfection is extremely rare. Congenital Rubella infection, unlike acquired infection, may cause disastrous clinical effects to the unborn child. A fetus may be stillborn or have such abnormalities as bone and cardiovascular defects, mental retardation, encephalitis, hepatomegaly, splenomegaly, thrombocytopenic purpura, cataracts and microcephaly. Because of severity of the complication from infection, detection in pregnant women is paramount. Therefore, it is important that the level of immunity be determined in women of child-bearing age, pregnant women, neonates who were exposed in utero, and others who may have been in close contact. The clinical recognition of Rubella infection is highly unreliable, and subclinical cases are frequent. Serological testing has been shown to be an effective method of detecting infection.

### PRINCIPLE OF THE TEST

The E-RVM-K15 Rubella IgM assay is based on the principle of the capture of these immunoglobulins and subsequent identification of those which are specific, making use of their ability to bind an antigen conjugated to peroxidase. The capture is performed using monoclonal antibodies bound to the solid phase (microtitration strips). The antigen is composed of purified and inactivated Rubella virus antigen.

### REAGENTS

The RD-RatioDiagnostics Rubella IgM ELISA kit contains sufficient reagents for 96 wells. Each kit contains the following reagents:

MATERIAL PROVIDED	QUANTITY	CATALOG NO.
Anti-human IgM Antibody-Coated Microtitration Strip	One Plate	E-ABM-10
Wash Concentrate	One Bottle	E-WSL-30
Sample Diluent	One Bottle	E-DLB-40
TMB-Substrate	One Bottle	E-TMB-08
Negative control	One Vial	E-RVM-01
Cut off control	One Vial	E-RVM-02
Positive control	One Vial	E-RVM-03
Rubella virus-HRP-Conjugate	One Bottle	E-RVM-20u
Stopping Solution	One Bottle	E-STP-09

### MATERIAL NOT PROVIDED

- Microtitration plate reader capable of absorbance measurement at 450 nm
- Deionized/Distilled water
- Precision pipette to deliver 10 µl, 100 µl, and 1 ml
- Semi-automatic pipette to deliver 100 µl
- Automatic microtitration plate washer
- Absorbent material for blotting the strips
- Incubator

## REAGENTS PROVIDED

### Antibody-Coated Microtitration Strips:

One strip holder containing 12x8 (96) microtitration wells coated with monoclonal Anti-human IgM antibodies. Store at 2-8°C until expiration date. Remove the support and strips to be used from the foil package, and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.

### Wash Concentrate:

One bottle, 100 ml, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% Brij weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.

### Sample Diluent:

One bottle, 100 ml, containing a BSA solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

### Rubella IgM Controls:

Three vials, each 2 ml of human serum in a 0.01 M phosphate buffer with BSA containing 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

### Rubellavirus-HRP- Conjugate:

One bottle, 12 ml, containing purified Rubella Virus antigen conjugated with peroxidase, in a phosphate buffer solution with 0.02% Proclin. Store at 2-8°C until expiration date.

### TMB-Substrate:

One bottle, 12 ml, containing tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer, pH 3.8. Store at 2-8°C until expiration date.

### Stopping Solution:

One bottle, 15 ml, containing 0.3 M H<sub>2</sub>SO<sub>4</sub> in solution. Store at 2-8°C until expiration date.

## PRECAUTIONS

For *in vitro* use

The following universal Good Laboratory Practices should be observed:

Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose all reagents and material in compliance with applicable regulations.

### WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

This kit may contain some reagents made with human and animal source material (e.g. serum, plasma or bovine albumin) or used in conjunction with human and animal source material. Human sera obtained from blood donors used in this kit have been tested by CE recommended methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HbsAg; the material from animal source is also free from infection. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 4<sup>th</sup> Edition, April 1999.

### WARNING AND PRECAUTION:

Some of the reagents in this kit contain sodium azide as a preservative at concentrations below the regulatory limit of < 0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes, and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin, eyes and clothing.

In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system.

For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

## SPECIMEN COLLECTION AND HANDLING

Serum should be used, and the usual precautions for venipuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at -20°C. Do not use hemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

## PREPARATION FOR ASSAY

*A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete washing will adversely affect the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed to add the TMB Chromogen Solution. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination of the TMB Chromogen Solution with the Conjugate. Use a clean disposable pipette tip for each reagent. Avoid pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and TMB can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.*

**PREPARATION OF REAGENTS:****Wash Solution:**

Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, they should be dissolved at 37°C before dilution. Pour 100 ml of the Wash Concentrate into a clean container and dilute by adding 900 ml of deionized/distilled water. Mix thoroughly by inversion. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.

**Microtitration Strips:**

Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

**Assay Procedure:**

All specimens and reagents to reach room temperature (~25°C) before use. Serum Samples and Controls should be assayed in duplicate. Cut-Off Serum should be assayed in triplicate.

1. Mark the microtitration strips to be used.
2. Dilute serum samples 1:101 distributing 10 µl of serum into 1 ml of Sample Diluent.
3. Pipette 100 µl of each diluted serum sample and controls to the appropriate well. Leave one well for the blank.
4. Incubate for 45 minutes at 37°C.
5. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.  
*NOTE: Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 0.35 ml of the Wash Solution into each well; (c) repeat step (a) and (b) four times.*
6. Add 100 µl of Rubella virus-HRP-Conjugate into each well.
7. Incubate for 45 minutes at 37°C.
8. Aspirate and wash each well four times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
9. Add 100 µl of TMB Chromogen Solution to each well using a dispenser.
10. Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
11. Add 100 µl of Stopping Solution to each well using a dispenser.
12. Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 600 or 620 nm.

**RESULTS**

Calculate the mean absorbance for each control and unknown.

**Qualitative results:**

If the absorbance of the sample is higher than that of the Cut-Off, the sample is positive for the presence of specific IgM.

Calculate the ratio between the average OD value of the sample and that of the Cut-Off. The sample is considered:

Positive: if the ratio is > 1.1.

Doubtful: if +/- 10% of the Cut-Off.

Negative: if the ratio is < 0.9.

If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

**LIMITATIONS OF THE PROCEDURE**

- A serum sample obtained during the late phase of infection, when only IgG antibodies are present, may be negative by this procedure.
- The test result should be used in conjunction with information available from the evaluation of other clinical and diagnostic procedures.
- Avoid repeated freezing and thawing of reagents and specimens.
- Grossly hemolyzed, icteric or lipemic specimens should be avoided.
- Heat inactivated sera should be avoided.
- Serological data of immunocompromised patients and newborn children have restricted value.

**QUALITY CONTROL**

Subtract the value of the blank from all the other readings. The OD values of Cut off control must be at least 0.2. Positive control must have an OD at least 1.5 times that of Cut off control.

## PERFORMANCE CHARACTERISTICS

### 1. Sensitivity and Specificity

154 well selected human sera, obtained from a clinical laboratory in Frankfurt, were analyzed by this Rubella IgM Elisa and reference Elisa method. Out of 154 samples, 17 were positive for the presence of IgM antibodies to Rubella virus by RD-RatioDiagnostics Elisa, and reference method also showed 17 of them as positive. The results are summarized below ( see Tab.1 ).

Tab1.

Assay Comparison	RD-RatioDiagnostics		
	Positive	Negative	Brd. Line
<i>TEST A</i>			
Positive	17	0	0
Negative	0	137	0
Brd Line	0	0	0

### 2. Precision

Tab.2

2. Inter-assay Study			
No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean	0.044	0.7	1.07
SD	0.002	0.01	0.02
CV%	4.9	1.9	1.5

Tab.3

3. Intra-assay study			
No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean	0.036	0.55	0.91
SD	0.003	0.028	0.023
CV%	9.6	5.2	2.5

### 3. Interferences

Testing of potentially cross reactive sera to IgM assays, as like rheumatoid factor (RF) and Infectious Mononucleosis (EBV), revealed no false test results for RD-RatioDiagnostics Rubella IgM Elisa. However, complete eliminating of cross reaction with EBV cannot be assured.

Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 5 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

## REFERENCES

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4. Mahony, JB, and Chernesky, MA: Rubella virus. In Rose, NR, et al (eds): Manual of Laboratory Immunology, ed. 4. ASM, Washington, DC, 1992, pp. 600-605.