

RUBELLA IgM ELISA Kit

96 Tests Kit

Enzyme Immunoassay for the Detection of
specific RUBELLA IgM Antibody
(For In Vitro Diagnostic Use Only)
Catalogue No. PT-Rub-IgM-96

PISHTAZ TEB DIAGNOSTICS

Introduction

RUBELLA virus is classified as a togavirus, and the only member of Rubivirus genus. The virus consists of single strand RNA, inner icosahedral nucleocapsid and lipoprotein envelope. RUBELLA (German measles, 3 days measles) is an acute febrile illness which displays various sign and symptoms like skin rash, posterior auricular and suboccipital lymphadenopathy. Among viral infection with skin rash manifestation, RUBELLA virus cause mildest disease. However, in pregnancy the virus cause Congenital RUBELLA Syndrome (CRS) which accompanies serious fetal complication based on gestational age at the time of infection. Maternal infection during early pregnancy (first trimester) leads to congenital abnormalities in 85% of neonates. This percentage decline to 15% if infection occurs during second trimester. Mental retardation, heart disease, cataract, deafness, meningoencephalopathy and progressive pan-encephalitis are among fetal abnormalities caused by RUBELLA virus. Infection (Viremia) during third trimester tends to no obvious symptoms in fetus.

Detection of specific anti RUBELLA IgM corresponds to immunity against the virus. Diagnosis of RUBELLA infection during pregnancy is crucial. To diagnose RUBELLA infection reliably, two separate serum samples taken 10 days apart are used to demonstrate specific anti-RUBELLA IgM titer rising. Demonstration of specific anti-RUBELLA IgM antibody in individual sample is the alternative serological technique for diagnosis of RUBELLA infection. Hemagglutination inhibition (HI) and ELISA are considered as standard techniques for diagnosis of RUBELLA infection. Since HI method needs pretreatment to remove nonspecific inhibitors, ELISA method preferred mostly.

Test Principle

The test principle is based on antibody capture ELISA technique in which microplate wells is coated with anti-human IgM antibody. Diluted patient serum samples are allowed to react with coated antibodies. After incubation, All serum IgM molecules will attached to the coated antibodies and unbound IgM antibodies are removed by washing. Then, to find specific anti-Rubella IgM, conjugated HRP-Rubella antigen added into wells which bound to their specific IgM antibody. After incubation and appropriate washing, a solution of chromogen substrate is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of RUBELLA IgM is directly proportional to the color intensity of the test sample.

Materials Provided With Kit

1. Antigen coated plate (1 plate, 96 wells): Microtiter wells coated with anti-human IgM antigens.
2. Enzyme Conjugate: 1 vial, 12 ml volume. The solution contains HRP conjugated Rubella antigen, buffer and preservative. Ready to use.
3. Sample diluent: 2 vials each contains 50 ml of diluent to dilute samples. The solution contains buffer, protein and stabilizer, ready to use.
4. Positive serum control (Red): 1 vial, 1.5 ml solution containing protein as stabilizer, 0.05% Kathon CG as preservative and inactivated Rubella IgM positive human serum.
5. Cut off serum control (Blue): 1 vial, 1.5 ml volume, containing protein as stabilizer, 0.05% Kathon CG as preservative and inactivated Rubella IgM positive human serum.
6. Negative control serum (Yellow): 1 vial, 2 ml, buffer solution containing 0.05% Kathon CG as preservative and negative for Rubella IgM human serum.
7. Wash solution (20X): 1 vial, 50 ml, Phosphate Buffer Saline (PBS) containing 0.05 % Tween 20 as detergent and Kathon CG as preservative.

8. Chromogen-substrate: 1 vial, 12 ml, contains tetramethyl benzidine (TMB) and hydrogen peroxide, ready to use.
9. Stop solution: 1 vial, 12 ml, 1 molar hydrochloric acid solution.
10. Cardboard sealer.

Materials/Equipment required but not provided with Test Kit

1. ELISA reader with 450 and 630 nm (reference) filters.
2. Precision pipette (10 and 100 µl).
3. Distilled water.
4. Absorbent paper.
5. 37 °C Incubator or water bath.

General Information

1. Do not mix kit reagents from different lot numbers. All kit components must be used only in original kit.
2. This kit is just for the detection of Anti-RUBELLA IgM in human serum and plasma.
3. All reagents obtained from human sources are negative for HIV Ag, HIV and HCV antibodies. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.

Storage Conditions

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep Un-used wells in their sealed bag with desiccants.
3. Opened microplates are stable for 4 months.
4. Reagents stability period are marked on their labes.

Specimen Collection and Preparation

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. If testing cannot be done within an hour after sample collection, refrigerate the specimen (maximum 2 days at 2-8°C) and let it

return to room temperature before testing. If prolong storage is required, samples should be stored at -20°C. Avoid freeze-thaw of specimen during storage. Samples suspected to microbial contamination should not be used.

Reagents & Specimens Preparation

1. All reagents should be allowed to reach room temperature (22-28°C) before use.
2. Working wash solution: Warm the vial at 37°C to dissolve possible crystals which formed due to concentration of solution. dilute concentrated wash solution 1:20 with distilled water before use. This solution will be stable for 1 week at 2-8 °C.

Assay Procedure

1. Use required number of wells and keep the remaining with desiccants in tightly closed sealed bag. Consider first well in first strip as **Blank**, next one for **Negative serum control**, next two wells for **Cut off** serum control, and another next one for **Positive control**. Test steps should be done sequentially.
2. Dilute samples 1:101 with sample diluent (dilute 10 µl of sample with 1 ml of sample diluent). Kit's control sera are ready to use and do not need any dilution.
3. Add 100 µl of each control serum (positive, negative and cut off) as well as diluted test sera into appropriate wells. Duplicate runs are suggested.
4. Seal the plate with cardboard sealer tightly. Mix gently and leave wells for 30 minutes at 37°C.
5. Remove the wells content by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times (each with 300 µl of Working wash solution).

6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
7. Add 100 µl of ready to use conjugate solution into all wells.
8. Seal the plate with cardboard sealer tightly. Mix gently and incubate wells for 30 minutes at 37°C.
9. Repeat steps of 5 and 6.
10. Add 100 µl of chromogen/ substrate solution to each well.
11. Incubate the microplate wells at room temperature and dark for 15 minutes to develop color.
12. Add 100 µl of stop solution to the wells to stop reaction.
13. Read absorbance at 450 nm by ELISA reader (Use 630 nm filter as reference filter if it's available) reference filter is highly recommended.)

Validity of the Assay

The assay is to be considered valid if:

1. The OD (450 nm) value of the **Blank** should be lower than 0.1. Higher values indicate chromogen/substrate contamination.
2. The OD value for the **Negative control serum** is lower than 0.15. Higher values indicate an inappropriate washing procedure. In such a case, check the efficiency of the washing device.
3. The **Cut off control serum** mean OD higher than 0.15.
4. The OD value of **Positive serum control** is higher than the 0.6. Lower values indicate kit reagents decay. In such a case, check expiry date of the kit before repeating the assay.

Result Calculation

1. Measure absorbance of controls and samples at 450 nm (Use 630 nm filter as reference filter if it's available).
2. Reduce blank OD from tests and controls OD.

To estimate Cut off value, Mean OD of Cut off control serum should be calculated:

Cut-off value = Mean OD of Cut-off control

To distinguish between positive and negative results the cut off index should be determined:

Cut-off Index = OD of sample/Cut-off value

Based on above formula, Results lower than 0.9 is considered as negative and those greater than 1.1 considered as positive results. Those results between 0.9-1.1 is considered as suspected results and should be re-evaluated with fresh samples after a while.

Results Evaluation

1. Negative results indicate absence of anti-Rubella IgM antibody.
2. Positive results should be rechecked and those which become negative should be reported as negative. Faulty washing and sampling errors may leads to first positive results.

Performance Characteristics

Sensitivity

To evaluate test sensitivity, 25 positive samples confirmed by chemiluminescence and ELISA were used which all showed positive results. The PT-RUBELLA-IgM-ELISA showed 100% sensitivity which is comparable to other diagnostic kits or methods.

Specificity

To evaluate test specificity, 200 negative sera are tested simultaneously with PT RUBELLA-IgM-ELISA and chemiluminescence method. Results displayed 197 negative and 3 positive results which in test repeat 2 became negative. According to test results the PT RUBELLA-IgM-ELISA showed 99% specificity.

Test Precision

To verify test repeatability, intra- and inter assay tests were performed on positive, weakly positive and negative sera. Results are shown below:

Intra-assay test results

	Number of Repeats	Mean OD	SD	CV%
Positive Sample	16	1.06	0.14	13.2
Negative Samples	16	0.08	0.003	3.7
Weak Positive	16	0.25	0.14	5.6

Inter-assay test results

	Number of Repeats	Mean OD	SD	CV%
Positive Sample	10	1.08	0.15	13.8
Negative Samples	10	0.08	0.004	5.0
Weak Positive	10	0.24	0.02	8.3

All tests were run in duplicate.




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References

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Schematic RUBELLA-IgM ELISA Test Procedure			
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Step 1




Reagent	Control Serum	Blank	Sample
			
Control Sera	100 µl	None	None
Diluted Sample	None	None	100 µl

Cover the microplate wells with cardboard sealer tightly, Mix gently for 15 seconds and incubate them for 30 minutes at 37°C.


Step 2

Remove plate cover and discard reagents of wells. Wash the microplate wells for 5 times according to test manual.


Step 3




Enzyme Conjugate Solution	100 µl	None	100 µl
			

Cover the microplate wells with cardboard sealer and incubate for 30 minutes at 37°C.


Step 4




Remove plate cover and discard reagents of wells. Wash the microplate wells for 5 times according to test manual.


Step 5

Chromogen Substrate solution	100 µl	100 µl	100 µl
			

Incubate wells for 15 minutes at room temperature and dark.

Step 6

Stop Solution	100 µl	100 µl	100 µl
			

Read absorbance at 450 nm (Use 630 nm filter as reference filter if it's available).