

## RUBELLA IgG ELISA Kit

96 Tests Kit

Enzyme Immunoassay for the  
Detection of RUBELLA IgG Antibody  
(For In Vitro Diagnostic Use Only)  
Catalogue No. PT-Rub IgG-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 display 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 IgG 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 IgG 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 indirect ELISA technique in which diluted patient serum samples are allowed to react with coated RUBELLA antigens. After incubation, the HRP -conjugated anti-human IgG is added into the wells. If anti-RUBELLA IgG was present in samples they will react with the HRP-conjugated anti-human IgG. 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 IgG 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 RUBELLA antigens.
2. Sample diluent: 2 vials each contains 50 ml of diluent to dilute samples. The solution contains buffer, protein and stabilizer, ready to use.
3. Conjugate solution: 1 vial, 12 ml volume. The solution contains HRP conjugated anti-human IgG, buffer and preservative. Ready to use.
4. Standards set: 5 vials, each contains 1.5 ml of standard solution including 0, 10, 50, 100, 200 IU/ml of anti-RUBELLA-IgG standard calibrated against WHO reference standards.
5. Wash solution: 1 vial, 50 ml (20X), Phosphate Buffer Saline (PBS) containing 0.05 % Tween 20 as detergent and Kathon CG as preservative.
6. Chromogen-substrate: 1 vial, 12 ml, contains tetramethyl benzidine (TMB) and hydrogen peroxide. Ready to use.
7. Stop solution: 1 vial, 12 ml, 1 molar hydrochloric acid solution.
8. Cardboard sealer.

### Materials/Equipment required but not provided with Test Kit

1. ELISA reader with 450 and 630 nm (reference) filters.
2. Precision pipettes (10 and 100 µl).
3. Distilled water.

- Absorbent paper.

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.

## General Information

- Do not mix kit reagents from different lot numbers. All kit components must be used only in original kit.
- This kit is just for the detection of Anti-RUBELLA IgG in human serum and plasma.
- 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

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

- All reagents should be allowed to reach room temperature (22-28°C) before use.
- Working wash solution: Warm the vial at 37°C to dissolve possible crystals

## Assay Procedure

- Use required number of wells and keep the remaining with desiccants in tightly closed sealed bag. Test steps should be done sequentially.
- Dilute samples 1:101 with sample diluent (dilute 10 µl of sample with 1 ml of sample diluent). Standards set are ready to use and do not need any dilution.
- Add 100 µl of each standard as well as diluted test sera into appropriate wells. Duplicate runs are suggested.
- Seal the plate with cardboard sealer tightly. Mix gently and leave wells for 30 minutes at room temperature (22-28°C).
- 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).
- Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
- Add 100 µl of ready to use conjugate solution into all wells.
- Seal the plate with cardboard sealer tightly. Mix gently and incubate wells for 30 minutes at room temperature (22-28°C).
- Repeat steps of 5 and 6.
- Spike 100 µl of chromogen/ substrate solution to each well.
- Incubate the microplate wells at room temperature and dark for 15 minutes to develop color.
- Add 100 µl of stop solution to the wells to stop reaction.
- 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 mean OD (450 nm) of the zero standard should be lower than 0.1. Higher values indicate inappropriate washing or chromogen/substrate contamination. In such a case, repeat the assay with particular attention into washing steps, the efficiency of the washing device and verify the chromogen color which should be colorless.
2. The mean OD value of standard 10 (the second standard) should be higher than zero standard.
3. The mean OD value of standard 200 should be higher than 1.5. Lower values indicate kit reagents decay. In such a case, check expiry date of the kit before repeating the assay.

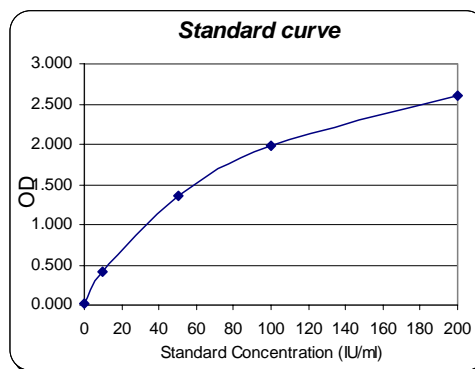
## Result Calculation

1. Measure absorbance of Standards and samples at 450 nm (Use 630 nm filter as reference filter if it's available).
2. Construct a point to point standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in IU/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Use the mean absorbance value for each sample, determine the corresponding concentration of Anti-RUBELLA IgG in IU/ml from the standard curve.

In normal population, the cut-off value considered as 10 IU/ml. Results lower than 10 IU/ml is considered as negative and those greater than 10 IU/ml considered as positive results. Those results between 9-11 IU/ml is considered as suspected results and should be re-evaluated with fresh samples after a while.

## Example of Standard curve

Standards (IU/ml)	Absorbance
0	0.025
10	0.419
50	1.361
100	1.987
200	2.606



Note: The standard curve shown in above example is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

## Performance Characteristics

### - Sensitivity

To evaluate test sensitivity, 200 positive samples confirmed by chemiluminescence and ELISA were used which all showed positive results. The PT-RUBELLA-IgG-ELISA showed 100% sensitivity which is comparable to other diagnostic kits or methods.

### Specificity

To evaluate test specificity, 100 negative sera are tested simultaneously with PT RUBELLA-IgG-ELISA and chemiluminescence method. Results displayed 99 negative and 1 positive results which in test repeat became negative. According to test results the PT RUBELLA-IgG-ELISA showed 100% 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:

- medical microbiology twenty second edition. Mc Grow-Hill 2001.
5. Murray patrick R.Rosenthal Ken S.Kobayashi George S.Medical Microbiology fourth Edition. Mosby2002.

**Intra-assay test results**

	<b>Number of Repeats</b>	<b>Mean Conc. (IU/ml)</b>	<b>SD</b>	<b>CV%</b>
<b>Negative Sample</b>	20	4.4	0.3	6.8
<b>Positive Samples</b>	20	108	4.9	4.5
<b>Weak Positive</b>	20	18	1	5.5

**Inter-assay test results**

	<b>Number of Repeats</b>	<b>Mean Conc. (IU/ml)</b>	<b>SD</b>	<b>CV%</b>
<b>Negative Sample</b>	10	4.5	0.4	8.8
<b>Positive Samples</b>	10	107	4.6	4.2
<b>Weak Positive</b>	10	18	1.2	6.6



All tests were run in duplicate.

**References**

- Centers for Disease Control and Prevention. Rubella and congenital Rubella syndrome - United States, 1994-1997. MMWR 1997;46:350-4.
- de Souza Va; Sumita LM; Otsubo ME; Takei K; Pannuti CS. Enzyme linked immunosorbant assay for Rubella antibodies:a sample method of antigen production. A preliminary report. Rev Inst Med Trop sao Paulo 1995;37(4):357-9.
- Engvali. E. and Perlmann. P.(1997) Enzyme linked immunosorbent assay (ELISA).Quantitative assay forimmunoglobulin. J.immuchochemistry ,8,871-874.
- Brooks Georf. Butel janet S.Jawetz,melnick&Adelbery`s

### Schematic RUBELLA-IgG ELISA Test Procedure

**Step 1**



Reagent	Standards	Sample
		
Standards	100 µl	None
Diluted Sample	None	100 µl

Cover the microplate wells with cardboard sealer tightly and incubate them for 30 minutes at room temperature (22-28°C).


**Step 2**

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

**Step 3**



Ready to use Conjugate Solution	 100 µl	 100 µl
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Cover the microplate wells with cardboard sealer and incubate for 30 minutes at room temperature (22-28°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
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Incubate wells for 15 minutes at room temperature and dark.


**Step 6**

Stop Solution	 100 µl	 100 µl
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Read absorbance at 450 nm (Use 630 nm filter as reference filter if it's available).