

**RUBELLA IgM
ELISA Kit Protocol**

(Cat. No.:EK-310-83)

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INTENDED USE

The Rubella IgM ELISA is intended for use in the detection of IgM antibody to the rubella virus. **FOR RESEARCH ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES**

WARNINGS AND PRECAUTIONS FOR USERS

1. Potential biohazardous materials:
The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. This test kit is designed for investigational use.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components from different lots should not be mixed.
5. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

TABLE OF CONTENT

Introduction and Protocol Overview	4
Principle of the Test	4
Storage	5
List of Components	5
Specimen Collection and Preparation	5
Reagent Preparation	6
Assay Procedure	6
Calculation of Results	7
Interperation	8
Performance Characteristics	8
References	9

INTRODUCTION AND PROTOCOL OVERVIEW

Rubella is a herpes virus. Generally rubella is considered a mild adolescent disease. However, a maternal infection can be transmitted through the placenta to the fetus, causing congenital rubella. Congenital rubella may result in chronic cardiac disease, growth retardation, hepatosplenomegaly, malformations and other severe anomalies. Children born asymptomatic may develop these abnormalities later in life. To reduce the risk of such severe complications, accurate serological methods must be performed to determine the serologic status of childbearing-aged women. A woman tested to be non-immune can be educated on infection and differentiate rubella from other exanthematous diseases. Expecting women with current rubella infection should be counseled on the consequences of congenital infection.

PRINCIPLE OF THE TEST

Purified Rubella antigen is coated on the surface of microwells. Diluted patient serum is added to the wells, and the Rubella IgM- specific antibody, if present, binds to the antigen during incubation. After washing the wells to remove unbound sample, antibody to human IgM conjugated with horse-radish peroxidase (HRP) is added and incubated at 37°C for 30 minutes. Unbound conjugate is removed by a subsequent washing step. A solution of TMB Reagent is then added to the microwells. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgM-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

CAUTION: Phoenix Pharmaceuticals guarantees that its products conform to the information contained in this publication. The purchaser must determine the suitability of the product for its particular use and establish optimum sample concentrations.

STORAGE

1. Store the kit at 4°C. **DO NOT FREEZE.**
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

LIST OF COMPONENTS

Materials Provided with the Kit:

- Microtiter Wells: purified Rubella antigen coated wells (12 x 8 wells)
- Enzyme Conjugate Reagent (red color): Red cap. 1 vial (12 ml)
- Sample Diluent (blue color): Natural cap. 1 bottle (22 ml)
- Negative Control: Range stated on label. Natural cap (150 µL/vial)
- Cut-off Calibrator: Yellow cap. Rubella IgM Index = 1 (150 µL/vial)
- Positive Control: Range stated on label. Red cap. (150 µL/vial)
- Wash Buffer Concentrate (20x): Natural cap. 1 bottle (50 ml)
- TMB Reagent (One-Step): 1 vial (11 ml)
- Stop Solution: 1N HCl, Natural cap. 1 vial (11 ml)

SPECIMEN COLLECTION AND PREPARATION

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 4°C for up to 7 days or frozen for up to 6 months. Avoid repetitive freezing and thawing of serum sample.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25 °C) before use.
2. Dilute 1 volume of Wash Buffer (20x) with 19 volumes of distilled water. For example, dilute 50 ml of Wash Buffer (20x) into distilled water to prepare 1000 ml of Wash Buffer (1x). Wash Buffer is stable for 1 month at 4°C. Mix well before use.

ASSAY PROCEDURE

Note: *Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-25°C).*

1. Place the desired number of coated wells into the holder.
2. Prepare 1:40 dilution of test samples, Negative Control, Positive Control, and Calibrator by adding 5 µl of the sample to 200 µl of Sample Diluent. Mix well.
3. Dispense 100 µl of diluted sera, Calibrator, and Controls into the appropriate wells. For the reagent blank, dispense 100 µl Sample Diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well.
4. Incubate at 37°C for 30 minutes.
5. At the end of incubation period, remove liquid from all wells. Rinse and flick the microtiter wells 5 times with diluted Wash Buffer (1x).
6. Dispense 100 µl of Enzyme Conjugate to each well. Mix gently for 10 seconds.
7. Incubate at 37°C for 30 minutes.
8. Remove Enzyme Conjugate from all wells. Rinse and flick the microtiter wells 5 times with diluted Wash Buffer (1x).
9. Dispense 100 µl of TMB Reagent into each well. Mix gently for 10 seconds.
10. Incubate at 37°C for 15 minutes.
11. Add 100 µl of Stop Solution (1N HCl) to stop reaction.
12. Mix gently for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely**
Note: Make sure there are no air bubbles in each well before reading.
13. Read O.D. at 450 nm **within 15 minutes** with a microwell reader.

CALCULATION OF CUT-OFF RESULTS

1. Calculate the mean of duplicate cut-off calibrator value xc.
2. Calculate the mean of duplicate positive control (xp), negative control (xn) and patient samples(xs).
3. Calculate the Rubella IgM Index of each determination by dividing the mean values of each sample (x) by calibrator mean value, xc.

Example of typical results: Note: The O.D. values are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data.

Cut-off Calibrator Rubella IgM Index = 1.0

1. Cut-off Calibrator O.D. = 0.791, 0.747 xc = 0.769
2. Negative Control O.D. = 0.065,0.071 xn = 0.068
Rubella IgM Index = $xn / xc = 0.068 / 0.769 = 0.09$
3. Positive Control O.D. = 1.488, 1.545 xp = 1.517
Rubella IgM Index = $xp / xc = 1.517 / 0.769 = 1.97$
4. Patient sample O.D. = 1.029, 1.046 xs = 1.038
Rubella IgM Index = $xs / xc = 1.038 / 0.769 = 1.35$

SUMMARY OF ASSAY PROCEDURE

1. Sample dilution 1:40
5 µl / 200 µl
2. Three incubations at 37°C

Diluted Sample	Enzyme Conjugate	TMB Reagent (One-Step)
100 µl	100 µl	100 µl
30 min.	30 min.	15 min

3. Stop with 100 µl of acid. Read O.D. at 450 nm

RUBELLA IgM ELISA KIT PROTOCOL

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.250.
2. If the O.D. value of the Cut-off Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The Rubella IgM Index for Negative and Positive Controls should be in the range stated on the Certificate of Analysis.

INTERPRETATION

- Negative: Rubella IgM Index less than 0.90 is negative for IgM antibody to Rubella virus.
- Equivocal: Rubella IgM Index between 0.91-0.99 is equivocal. Sample should be retested.
- Positive: Rubella IgM Index of 1.00 or greater is positive for IgM antibody to Rubella virus. It is indicative of acute rubella infection in a time of zero to three months before the blood samples were obtained.

PERFORMANCE CHARACTERISTICS

1. Specificity and Sensitivity:

A total of 159 patient samples were used to evaluate specificity and sensitivity of the test. The Rubella IgM ELISA test results were compared to the results of a commercial ELISA kit.

Reference Rubella IgM ELISA					
		N	E	P	Total
Ru- bella IgM ELISA	N	140(D)	2	1(B)	143
	E	0	0	0	0
	P	1 (C)	0	15 (A)	16
Total		141	2	16	159

$$\text{Sensitivity} = A / (A+B) = 15 / 16 = 93.8\%$$

$$\text{Specificity} = D / (C+D) = 140 / 141 = 99.3\%$$

$$\text{Accuracy} = (A+D) / (A+B+C+D) = 155 / 157 = 98.7\%$$

2. Precision

a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of four dif-

RUBELLA IgM ELISA KIT PROTOCOL

ferent serum samples in one assay. Within-assay variability is shown below:

Samples	1	2	3	4
# Replicates	24	24	24	24
Mean A450	2.324	1.027	0.458	0.123
S.D. (A450)	0.070	0.022	0.012	0.003
C.V. (%)	3.0	2.1	2.6	2.4

b. Inter-Assay Precision

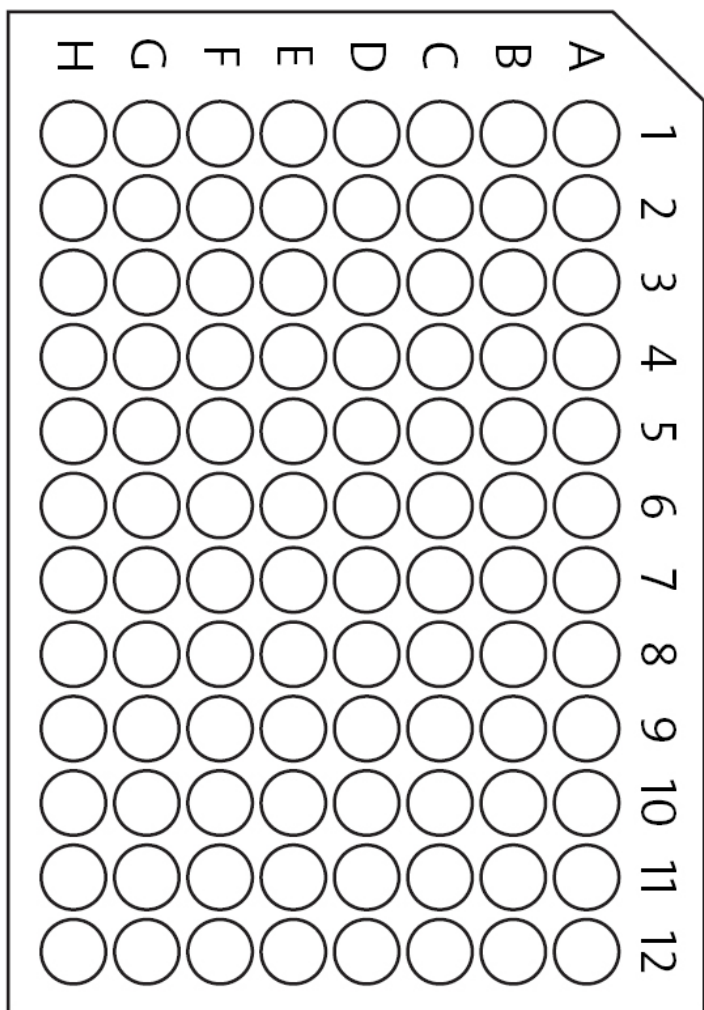
Between-run precision was determined by replicate measurements of four different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

Samples	1	2	3	4
# Replicates	20	20	20	20
Mean A450	2.373	1.022	0.472	0.141
S.D. (A450)	0.181	0.057	0.040	0.017
C.V. (%)	7.6	5.6	8.5	12.3

REFERENCES

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ASSAY DIAGRAM



NOTES