

# **ab178654 – Anti-Rubella Virus IgM Human ELISA Kit**

## Instructions for Use

For the qualitative measurement of IgM class antibodies against Rubella Virus in Human serum.

This product is for research use only and is not intended for diagnostic use.

# Table of Contents

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## INTRODUCTION

1. BACKGROUND	2
2. ASSAY SUMMARY	4

## GENERAL INFORMATION

3. PRECAUTIONS	5
4. STORAGE AND STABILITY	5
5. MATERIALS SUPPLIED	5
6. MATERIALS REQUIRED, NOT SUPPLIED	6
7. LIMITATIONS	6
8. TECHNICAL HINTS	7

## ASSAY PREPARATION

9. REAGENT PREPARATION	8
10. SAMPLE COLLECTION AND STORAGE	8
11. SAMPLE PREPARATION	8
12. PLATE PREPARATION	9

## ASSAY PROCEDURE

13. ASSAY PROCEDURE	10
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## DATA ANALYSIS

14. CALCULATIONS	12
15. TYPICAL SAMPLE VALUES	14
16. ASSAY ANALYTICAL SPECS	14

## RESOURCES

17. INTERFERENCES	15
18. TROUBLESHOOTING	15
19. NOTES	17

## 1. BACKGROUND

Abcam's anti-Rubella Virus IgM *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate qualitative measurement of IgM class antibodies against Rubella Virus in Human serum and plasma.

A 96-well plate has been precoated with anti-Human IgM antibodies to bind to corresponding antibodies in the sample. Controls or test samples are added to the wells and incubated. Following washing, a horseradish peroxidase (HRP) labelled Rubella Virus antigen conjugate is added to the wells, which binds to the captured Rubella Virus-specific IgM antibodies. TMB is then catalyzed by the HRP to produce a blue color product that changes to yellow after adding an acidic stop solution. The density of yellow coloration is directly proportional to the amount of Rubella Virus IgM sample captured in plate.

Rubella is an enveloped RNA virus belonging to the togaviruses. It has a spherical shape measuring about 50-70 nm in diameter. There appears to be only one antigenic type, and no cross-reactivity with alphaviruses or other members of the togavirus group has been found. Rubella viruses are pathogens of the respiratory tract and transmitted mainly by droplet infection. Rubella is a worldwide common contagious disease with mild constitutional symptoms and a generalized rash. In childhood, it is an inconsequential illness, but when it occurs during pregnancy, there is a significant risk of severe damage to the fetus. The risk of congenital rubella depends primarily on the month of pregnancy in which infection is acquired: overall, app. 16% of infants have major defects at birth following maternal rubella in the first 3 months of pregnancy. Congenital rubella infection may lead to a syndrome with single or multiple organ involvements, known as embryopathy rubeolosa. In some cases infection is inapparent but results in consequential damages as eye defects, deafness, growth retardation, and others. Naturally acquired immunity usually is long-lasting, but reinfection is possible due to decreasing levels of circulating antibodies. For immunization a vaccine containing live virus is used.

Species	Disease	Symptoms	Mechanism of Infection
Rubella Virus	Acquired rubella (german measles)	Generalized rash (fever, nausea)	Transmission by close person-to-person contact, spread most probably by droplets via the respiratory tract
	Congenital rubella syndrome (Embryopathia rubeolosa)	Cardiovascular lesions, eye defects, hearing impairment, CNS involvement and others	Fetal infection: transmission by hematogenous spread during maternal viremia

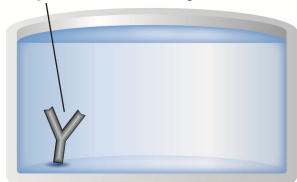
Infection may be identified by

- PCR
- Hemagglutination inhibition (HAI), Haemolysis-in-gel test
- (HiG)Serology: Detection of antibody production by ELISA

Measurement of antibodies in the serum is important for the determination of the immune status. Even a previous infection though rather overt may not yield a long-lasting immunity, but may result in an antibody titer too low to prevent reinfection. Especially the screening of adolescents and young women should be a mandatory routine in prenatal care.

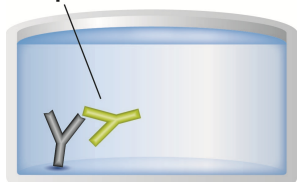
## 2. ASSAY SUMMARY

**Capture Antibody**



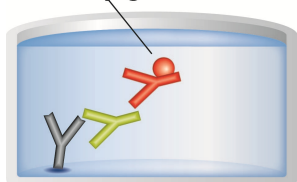
Prepare all reagents, samples and standards as instructed.

**Sample**



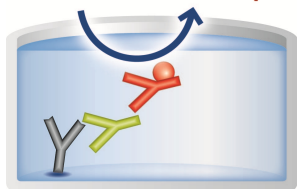
Add control IgMs and samples to each well used. Incubate at 37°C.

**HRP Conjugate**



Wash then add prepared HRP conjugated Rubella virus antigen to each well. Incubate at room temperature.

**Substrate      Colored product**



Wash the add TMB substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

## 3. PRECAUTIONS

**Please read these instructions carefully prior to beginning the assay.**

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

## 4. STORAGE AND STABILITY

**Store kit at 2-8°C immediately upon receipt.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

## 5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Anti-Human IgM Coated Microplate (12 x 8 wells)	96 Wells	2-8°C
IgM Sample Diluent***	100 mL	2-8°C
Stop Solution	15 mL	2-8°C
20X Washing Solution*	50 mL	2-8°C
Rubella Virus HRP Conjugate**	12 mL	2-8°C
TMB Substrate Solution	15 mL	2-8°C
Rubella Virus IgM Positive Control***	2 mL	2-8°C
Rubella Virus IgM Cut-off Control***	3 mL	2-8°C
Rubella Virus IgM Negative Control***	2 mL	2-8°C
Strip Holder	1 unit	2-8°C
Cover Foil	1 unit	2-8°C

\* Contains 0.1 % Bronidox L after dilution

\*\* Contains 0.2 % Bronidox L

\*\*\* Contains 0.1 % Kathon

### 6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Incubator at 37°C
- Multi and single channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Timer

### 7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use

- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells

### 8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**



## 9. REAGENT PREPARATION

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use.

### 9.1 1X Washing Solution

Prepare 1X Washing Solution by diluting 20X Washing Solution with deionized water. To make 200 mL 1X Washing Solution combine 10 mL 20X Washing Solution with 190 mL deionized water. Mix thoroughly and gently.

- All other solutions are supplied ready to use

## 10. SAMPLE COLLECTION AND STORAGE

- Use Human serum samples with this assay. If the assay is performed within 5 days of sample collection, the specimen should be kept at 2-8°C; otherwise it should be aliquoted and stored deep-frozen (-20 to -80°C). If samples are stored frozen, mix thawed samples well before testing.

*Avoid repeated freezing and thawing.*

Heat inactivation of samples is not recommended

## 11. SAMPLE PREPARATION

- Before assaying, all samples should be diluted 1:100 with IgM Sample Diluent. Add 10 µL sample to 990 µL IgM Sample Diluent to obtain a 1:100 dilution. Mix gently and thoroughly.

## 12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates)

### **13. ASSAY PROCEDURE**

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **Please read the test protocol carefully before performing the assay. Reliability of results depends on strict adherence to the test protocol as described.**
- **If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300  $\mu$ L to 350  $\mu$ L to avoid washing effects.**
- **All controls (Rubella Virus IgM Positive, Rubella Virus IgM Negative and Rubella Virus IgM Cut-off) must be included with each assay performed to determine test results.**
- **Assay all standards, controls and samples in duplicate.**
  - 13.1. Prepare all reagents, standards, and samples as directed in the previous sections.
  - 13.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
  - 13.3. Add 100  $\mu$ L of controls and diluted samples into appropriate wells. Leave one well for substrate blank.
  - 13.4. Cover wells with the foil supplied in the kit and incubate for 1 hour at 37°C.
  - 13.5. Remove the foil, aspirate the contents of the wells and wash each well three times with 300  $\mu$ L of 1X Washing Solution. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 sec. After the last wash, remove the remaining 1X Washing Solution by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.  
Note: Complete removal of liquid at each step is essential for good assay performance.

- 13.6. Add 100  $\mu$ L Rubella Virus HRP Conjugate into all wells except for the blank well. Cover with foil.
- 13.7. Incubate for 30 minutes at room temperature. Do not expose to direct sunlight.
- 13.8. Repeat step 13.5.
- 13.9. Add 100  $\mu$ L TMB Substrate Solution into all wells
- 13.10. Incubate for exactly 15 minutes at room temperature in the dark.
- 13.11. Add 100  $\mu$ L Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.  
Note: Any blue color developed during the incubation turns into yellow.
- 13.12. Measure the absorbance of the specimen at 450 nm within 30 minutes of addition of the Stop Solution.

*Dual wavelength reading using 620 nm as reference wavelength is recommended.*

## 14. CALCULATIONS

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate blank:** Absorbance value < **0.100**
- **Negative control:** Absorbance value < **cut-off**
- **Cut-off control:** Absorbance value **0.150 – 1.300**
- **Positive control:** Absorbance value > **cut-off**

If these criteria are not met, the test is not valid and must be repeated.

### Calculation of Results

Calculate the mean background subtracted absorbances for each sample and compare to mean Cut-off control value.

The Cut-off control value is the mean absorbance value of the Cut-off control wells.

Example: Absorbance value Cut-off control Well 1 = 0.156

Absorbance value Cut-off control Well 2 = 0.168

Mean Cut Off value:  $(0.156 + 0.168)/2 = 0.162$

### Results in Standard Units

Sample (mean) absorbance value x 10 = Standard Units  
Cut-off

Example:  $\frac{1.786 \times 10}{0.38} = 47 \text{ Standard Units}$

### Interpretation of Results

Negative: < 9 Standard Units

Positive: > 11 Standard Units

Grey zone: 9-11 Standard Units

Samples with an abundance between 9 and 11 NTU can not be considered as clearly positive or negative inconclusive (grey zone). It is recommended to confirm the results by testing the sample again in duplicate. If results in the second test are again in the grey zone a second serum sample should be tested and judged for a change in result.

## 15. TYPICAL SAMPLE VALUES

### PRECISION –

	Intra-Assay		Inter-Assay	
	Serum #1	Serum #2	Serum #3	Serum #4
n=	23	24	3	3
Mean	1.3	1.1	28.4	25.3
%CV	2.7	4.0	10.3	11.5

## 16. ASSAY ANALYTICAL SPECS

### SPECIFICITY -

The specificity is 98 % and is defined as the probability of the assay scoring negative in the absence of the specific analyte.

Cross reactivity with the different serum samples containing antibodies to Parvovirus, Chagas, Trichinella, Helicobacter, Toxoplasmosis, Mumps, EBV, TBE, Dengue virus, Mycoplasma and Bordetella is not observed.

### SENSITIVITY -

The sensitivity is 100 % and is defined as the probability of the assay scoring positive in the presence of the specific analyte.

## 17. INTERFERENCES

Interferences with hemolytic or lipemic sera are not observed.

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.

## 18. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time too short	Try overnight incubation at 4 °C
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)



## RESOURCES

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

### 19. NOTES



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