



RUBELLA VIRUS

IGM CLASS
u-capture assay

96 WELL ELISA KIT

PACKAGE INSERT
INSTRUCTIONS AND INFORMATION

Store at 2-8°C

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1. INTENDED USE

This kit is an *in-vitro* laboratory assay for the determination of IgM class Antibodies against Rubella Virus.

2. INTRODUCTION

Rubella is a spherical shape of about 50-70nm in diameter of an enveloped RNA virus belonging to the toga viruses. It is not known to cross react with other toga viruses or alpha viruses.

It is a pathogen of the respiratory tract and appears to be transmitted by droplet infection. It is a common contagious disease which presents with a generalised rash and mild constitutional symptoms. In children it is an inconsequential illness, however if infection is during pregnancy there is potential for severe damage to the foetus.

Congenital rubella infection during the first three months of pregnancy have a higher chance of major birth defects. In some cases infection may be undetected, but results in defects such as deafness, growth retardation, eye defects and others.

Naturally acquired immunity appears to be long lasting however reinfection may be possible due to decreasing levels of circulating antibody.

This is a vaccine preventable disease and the measurement of antibodies is important to determine immune status, especially for prenatal patients.

3. PRINCIPLE OF THE TEST

The Rubella Virus antibody test is an IgM u-capture immunoassay that detects Rubella specific IgM in human serum.

Anti-human IgM (coated onto microwells) bind corresponding antibodies in patient's serum. Anti-Horseradish peroxidase (HRP) labelled Rubella virus antigen is added and binds to immobilized Rubella specific antibodies.

This complex is then reacted with a specific substrate, Tetramethylbenzidine (TMB), to yield a blue colour. The intensity of the colour is proportional to the amount of Rubella-specific IgM antibodies in the serum. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour.

Following termination of colour development, the absorbance is measured at 450nm and reference at 620nm.

4. KIT COMPONENTS

1. Anti-human IgM class antibodies coated onto wells (IgM): 12 breakapart 8-well snap-off strips; vacuum sealed, in resealable aluminium foil.
2. Sample Diluent ***: 2 bottles containing 50 ml of buffer for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; Green cap.
3. Rubella Virus IgM Positive Control****: 1 bottle containing 2 ml; coloured yellow; ready to use; red cap.
4. Rubella Virus IgM Cut-off Control****: 2 bottle containing 1.5 ml; coloured yellow; ready to use; yellow cap.
5. Rubella Virus IgM Negative Control***: 1 bottle containing 2 ml; coloured yellow; ready to use; blue cap.
6. Conjugate** : 1 bottles containing 12 ml of peroxidase labelled antibody to human IgM; coloured yellow, ready to use; red cap.
7. Washing Solution (20x conc.)*: 1 bottle containing 50 ml of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.
8. TMB Substrate Solution: 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; amber cap. Colourless solution.
9. Stop Solution: 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; clear cap

* contains 0.01 % Kathon after dilution

** contains 0.2 % Thiomersal

*** contains 0.1 % Kathon

**** contains 0.02% Bronidox L and 0.02% Kathon

10. 1 Strip holder

11. 1 Package Insert

5. OTHER EQUIPMENT REQUIRED BUT NOT SUPPLIED

This protocol has been validated for the Triturus™ Analyser

1. MICROWELL PLATE READER (capable of reading at 450 / 620 nm).
2. WASH BOTTLE - 500mL or suitable MICROWELL PLATE WASHER.
3. MEASURING CYLINDERS
4. MULTICHANNEL PIPETTE
5. REAGENT TROUGHS
6. VARIABLE VOLUME PIPETTORS (10µL – 2mL) AND DISPOSABLE TIPS
7. INCUBATOR 37°C
8. DISTILLED WATER
9. VORTEX TUBE MIXER
10. TIMER
11. DISPOSABLE TUBES

6. ASSAY PROCEDURE

- Review all instructions thoroughly before testing.
- Room temperature incubations should be performed at 20 – 24°C.
- All reagents, samples and controls should be brought to room temperature before use.
- Unused strips are to be returned to the foil pouch along with the desiccant and resealed.
- The kit components should not be left at room temperature for longer than the procedure requires. Store kits and kit components at 2 – 8°C when not in use.
- Kit components from different lot numbers should not be interchanged.
- The WASH BUFFER CONCENTRATE (20x) may crystallise at 4°C. Incubate in a 37°C water bath until crystals have dissolved.
- To ensure accurate quantification, it is essential that all pipettes used in the assay are calibrated and a fresh tip is used for cut-off, controls and samples.
- Specimens can be stored at 2-8°C for 5 days before testing. If the assay is not completed within this time then aliquot and freeze (-20 to -70°C). Avoid repeated freezing and thawing.
- This assay requires
 - 1 well for substrate blank
 - 1 well for negative control
 - 2 wells for cut-off
 - 1 well for positive control
- Once started, finish the assay without interruption.

1. Perform a 1/101 dilution on the test sera.

NB: CONTROLS ARE READY TO USE

2. Remove the required number of microwells from the sealed bag and place them in the holder. Ensure unused wells are resealed and stored at 4°C.
3. Pipette 100µl of diluted samples and ready-to-use controls to the appropriate wells. Remember to leave a substrate blank.
4. Incubate for 60 MINUTES ± 5 MINUTES AT 37±1°C
5. Dilute the WASH BUFFER CONCENTRATE (20x) 1/20 with distilled water. Place in either a wash bottle or plate washer reservoir.
6. Wash Procedure: Aspirate the samples from the wells and then fill all wells with diluted WASH BUFFER (350µL/well) soak for 20 seconds on the first wash. Repeat the above for 4 more cycles but with no soak time.
NB: The washing protocol needs to be programmed for a STRIP WASH not a plate wash.
7. Add 100µL of CONJUGATE to all microwells except the substrate blank.
8. Incubate the plate for 30 MINUTES ± 5 MINUTES at ROOM TEMPERATURE.
9. Wash the plate as in step 6 using a plate wash. NB no soak time is required in this wash step.
10. Add 100µL/well of TMB SUBSTRATE to all microwells. Incubate the plate at ROOM TEMPERATURE for 15 MINUTES.

11. Stop the reaction by adding 100µL/well of STOP SOLUTION to the microwells.
12. Read the plate at 450/620nm within 15mins of stopping the reaction
13. Calculate the corrected optical densities (O.D.) by subtracting the Blank O.D. from the mean O.D. of the controls and samples.

7. CALCULATION AND INTERPRETATION OF RESULTS

The CUT-OFF is the mean absorbance value of the Cut-off control OD's.

Samples are considered DETECTED if the absorbance value is higher than 10% over the cut-off.

Samples are considered NOT DETECTED if the absorbance value is lower than 10% below the cut-off.

Samples with an absorbance value 10% above or below the cut-off should not be considered as clearly DETECTED or NOT DETECTED but in a grey zone.

It is recommended to repeat the test again 2-4 weeks later with a fresh sample. If results in the second test are again in the grey zone the sample is to be considered NOT DETECTED.

If a unit result is required apply the following formula

$$\frac{\text{Patient (mean) OD} \times 10}{\text{Cut-off}} = ?? \text{ units}$$

The following values should be used as a guide, however it is recommended that each laboratory should establish its own ranges for its patient population and geographical area.

Cut-off :	10 units
Grey Zone:	9-11 units
NOT DETECTED:	<9 units
DETECTED :	>11 units

8. PRECAUTIONS

1. In compliance with article 1 paragraph 2b European directive 98/79/EC, the use of this in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the test kits with analysers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
2. All components containing material of human origin have been tested and found NOT DETECTED for HBsAg, anti-HCV and antibodies to HIV. They should still, however, be treated as though they were potentially infectious.
3. Do not substitute any component for the ones supplied with the kit.
4. All components that contain preservatives must have care exercised in handling/disposing of these products.
5. Heat inactivation or repeated freezing and thawing of serum samples may cause erroneous results.
6. Performing the assay at temperatures other than those stated may cause erroneous results.

7. Do not use components after the expiry date stated on the label.
8. Care should be taken to reduce microbial contamination of reusable kit components.
9. To avoid cross-contamination do not interchange screw caps of components.
10. It is recommended that external or "in-house" controls be included with each assay.

WARNING: In the used concentration Bronidox L has hardly any toxicological risk upon contact with skin and mucous membranes!

WARNING: Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

9. REAGENT STORAGE AND SHELF LIFE

- All kit components must be stored at 2 – 8°C. All reagents are stable until labelled expiration date when stored at 2 – 8°C.

10. SPECIMEN COLLECTION

- Blood should be collected by venepuncture.
- Specimens can be stored at 2-8°C for 5 days before testing. If the assay is not completed within this time then aliquot and freeze (-20 to -70°C). Avoid repeated freezing and thawing.

11. QUALITY CONTROL

- The Substrate Blank, Cut-off, Positive Control and Negative Control should be run each time the assay is performed.
- Substrate blank: Absorbance value lower than 0.300.
- Negative control: Absorbance value lower than 0.200.
- Cut-off control: Absorbance value between 0.150 and 1.300.
- Positive control: Absorbance value equal to or greater than the cut-off value.

12. LIMITATIONS

- As with other diagnostic test procedures, the results obtained serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.
- Data from immunocompromised patients and newborns have only restricted value.
- Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values
- Interferences with haemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/ml haemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

13. TECHNICAL PERFORMANCE

A. INTRA-ASSAY VARIATION

Sample No.	n	Mean	%CV
Pos sample	23	1.3	2.7
Pos sample	24	1.1	4.0

B. INTER-ASSAY VARIATION

Sample No.	n	Mean	%CV
Pos Sample	48	28.4	10.3
Pos Sample	54	25.3	11.5

- The diagnostic specificity is defined as the probability of the assay scoring a negative in the absence of the specific analyte.
It is 98 %.
- The diagnostic sensitivity is defined as the probability of the assay scoring a positive in the presence of the specific analyte.
It is >98 %.

14. REFERENCES

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15. LIMITED EXPRESS LIABILITY

The manufacturer makes no express warranty other than the diagnostic kit will measure IgM antibodies against Rubella Virus when used in accordance with the manufacturer's instructions. The use of the diagnostic kit for any other purpose or for the clinical diagnosis of a disease state is outside the intended use of this product.

The manufacturer disclaims any and all implied merchantability, fitness for use or implied utility for any other purpose. Any or all damages for failure of the diagnostic kit to perform according to its instructions are limited to the replacement value of the kit.

In some jurisdictions the law makes these disclaimers unenforceable and, accordingly all or part of the disclaimer may not apply to all users.



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