

CE



RUBELLA VIRUS

IGG CLASS

96 WELL ELISA KIT

**PACKAGE INSERT
INSTRUCTIONS AND INFORMATION**

Store at 2-8°C

Product Code: 200 0110
Document Number : KBK-0041
Issue No: 3

1. INTENDED USE

This kit is an *in-vitro* laboratory assay for the determination of IgG 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 indirect solid-phase enzyme immunoassay that detects Rubella specific IgG in human serum.

Rubella Virus antigens (coated onto microwells) bind corresponding antibodies in patient's serum, forming an IgG-Ag complex. Anti-human IgG conjugated with the enzyme horseradish peroxidase (HRP) is added and binds to immobilized IgG.

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 IgG 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. Rubella Virus Coated Wells (IgG): 12 breakapart 8-well snap-off strips coated with Rubella Virus antigen; vacuum sealed, in resealable aluminium foil.
2. IgG Sample Diluent ***: 2 bottles containing 50 ml of buffer for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; amber cap.
3. Rubella Virus IgG Standards*** 4 vials containing 2mL, coloured yellow, blue caps, ready to use
STDA 100 IU/mL
STDB 50 IU/mL
STDC 10 IU/mL
STDD 0 IU/mL
4. Rubella Virus anti-IgG conjugate** : 2 bottles containing 10 ml of peroxidase labelled antibody to human IgG; coloured blue, ready to use; blue cap.
5. 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.
6. TMB Substrate Solution: 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; amber cap. Colourless solution.
7. 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 % Bronidox L

*** contains 0.1 % 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

4. Incubate for 60 MINUTES \pm 5 MINUTES AT 37 \pm 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 (350 μ L/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 100 μ L of Rubella anti-IgG CONJUGATE to all microwells except the substrate blank.
8. Incubate the plate for 30 MINUTES \pm 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 100 μ L/well of TMB SUBSTRATE to all microwells. Incubate the plate at ROOM TEMPERATURE for 15 MINUTES.
11. Stop the reaction by adding 100 μ L/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

For quantitative results in IU/mL, plot the standards on Lin/Lin graph paper or using linear regression. Compare the OD 's of the patients to that of the standards and read the IU/mL..

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.

Detected	>15 IU/mL
Equivocal	10-15 IU/mL
Not detected	<10 IU/mL

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, and standards should be run each time the assay is performed.
- Substrate blank: Absorbance value lower than 0.200.
- Standard D : Absorbance value lower than 0.300

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
Std A	6	2.34	2.5
Std B	6	1.73	3.4
Std C	6	0.54	7.9

B. INTER-ASSAY VARIATION

Sample No.	n	Mean	%CV
Std A	6	2.19	6.3
Std B	6	1.64	4.6
Std C	8	0.54	7.6

- 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

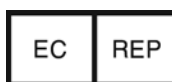
The manufacturer makes no express warranty other than the diagnostic kit will measure IgG antibodies against Rubella Virus when used in accordance with the manufacturer's instructions. The use of the diagnostic kit for any other purpose or for the clinical diagnosis of a disease state is outside the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