Version 22-04/24

User's Manual

Rubella virus IgG ELISA Kit

REF **DEIAPY61316**

Σ **96T**

RUO

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Enzyme immunoassay for the quantitative determination of IgG-class antibodies against Rubella Virus in human serumor plasma (citrate, heparin).

General Description

Rubella is an enveloped RNA virus belonging to the toga viruses. 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 alpha viruses or other members of the toga virus 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 foetus.

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 embryopathia 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.

The presence of pathogen or infection may be identified by:

1. PCR
2. Serology: e.g. ELISA, Hemagglutination inhibition (HAI)

Principles of Testing

The quantitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

Reagents And Materials Provided

1. Rubella Virus Coated Microplate (IgG): 12 break apart 8-well snap-off strips coated with Rubella Virus antigens; in resealable aluminium foil.
2. IgG Sample Diluent: 1 bottle containing 100 ml of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; colored yellow; ready to use; white cap.

3. Stop Solution: 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
4. Washing Buffer (20× conc.): 1 bottle containing 50 ml of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
5. Rubella Virus anti-IgG Conjugate: 1 bottle containing 20 ml of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); colored blue; ready to use; black cap.
6. TMB Substrate Solution: 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1%; ready to use; yellow cap; < 5% NMP.
7. Rubella Virus IgG Standards: 4 vials, each containing 2 ml standard (human serum or plasma); colored yellow; ready to use.

   Standard A: 0 IU/ml; blue cap

   Standard B: 10 IU/ml; green cap

   Standard C: 50 IU/ml; yellow cap

   Standard D: 100 IU/ml; red cap.

   The standards are calibrated in accordance with the WHO International Standard; Anti Rubella Immunoglobulin Human.
8. Cover foil: 1.
9. Instruction for use (IFU): 1.
10. Plate layout: 1.

    For potential hazardous substances please check the safety data sheet.

Materials Required But Not Supplied

1. ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
2. Incubator 37°C
3. Manual or automatic equipment for rinsing Microtiterplates
4. Pipettes to deliver volumes between 10 and 1000 µl
5. Vortex tube mixer
6. Distilled water
7. Disposable tubes

Storage

Store the kit at 2 - 8°C. The opened reagents are stable up to the expiry date stated on the label when stored at 2 - 8°C.

Specimen Collection And Preparation

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2 - 8°C; otherwise they should be aliquoted and stored deep-frozen (-70…-20°C). If samples are stored frozen, mix thawed samples well before testing. Avoid

repeated freezing and thawing. Heat inactivation of samples is not recommended.

Sample Dilution: Before assaying, all samples should be diluted 1+100 with Sample Dilution. Dispense 10 µl sample and 1 ml Sample Dilution into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

Reagent Preparation

It is very important to bring all reagents and samples to room temperature (20 - 25°C) and mix them before starting the test run!

Microtiterplate

The break-apart snap-off strips are coated with Rubella virus antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2 - 8°C.

Washing Buffer (20× conc.)

Dilute Washing Buffer 1+19; e.g. 10 ml Washing Buffer +190 ml distilled water. The diluted buffer is stable for 5 days at room temperature (20 - 25°C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

TMB Substrate Solution

The reagent is ready to use and has to be stored at 2 - 8°C, away from the light. The solution should be colorless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

Assay Procedure

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µl to 350 µl to avoid washing effects. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout. Select the required number of microtiter strips or wells and insert them into the holder.

•Perform all assay steps in the order given and without any delays.

•A clean, disposable tip should be used for dispensing each standard/control and sample.

•Adjust the incubator to 37 ± 1°C.

Steps

1. Dispense 100 µl standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1°C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on

tissue paper prior to the next step!

Note: Washing is important! Insufficient washing results in poor precision and false results.

5. Dispense 100 µl Conjugate into all wells except for the Substrate Blank well A1.
6. **Adjust tIncubate for 30 min at room temperature (20 - 25°C)**. Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20 - 25°C) in the dark**. A blue color occurs due to an enzymatic reaction.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a color change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

Measurement

1. Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank.**
2. If-due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!
3. **Measure the absorbance** of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.
4. Bichromatic measurement using a reference wavelength of 620 nm is recommended.
5. Where applicable calculate the **mean absorbance values** of all duplicates.

Calculation

Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

Substrate blank: Absorbance value < 0.100

Standard A: Absorbance value **< 0.200**

Standard B: Absorbance value **> 0.200**

Standard C: Absorbance value **> 0.700**

Standard D: Absorbance value **> 1.100**

Standard A < Standard B < Standard C < Standard D

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

Calculation of Results

In order to obtain quantitative results in IU/ml blot the (mean) absorbance values of the 4 Standards A - D on (linear/linear) graph paper in a system of coordinates against their corresponding concentrations (0, 10, 50 and 100 IU/ml) and draw a standard ccurve (absorbance values on the y-axis, concentrations on the x-axis).

Read results from this calibration curve employing the (mean) absorbance values of each patient sample.

For the calculation of the standard-curve mathematical Point to Point function should be used.

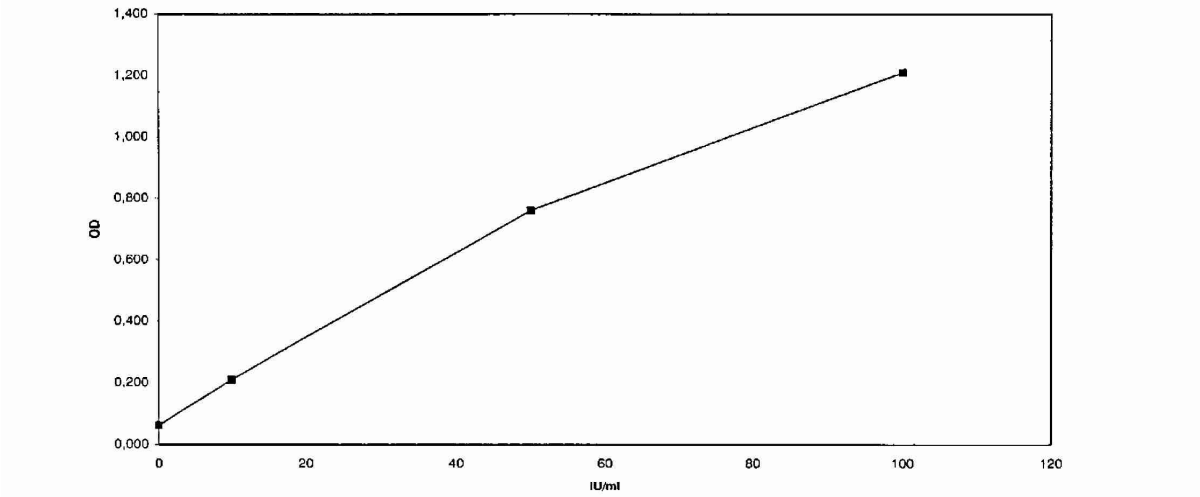
Interpretation Of Results

Normal value ranges for this ELISA should be established by each laboratory based on its own patient populations in the geographical areas serviced.

The following values should be considered as a guideline:

Positive	> 15 IU/ml	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	10 - 15 IU/ml	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative.**
Negative	< 10 IU/ml	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

Typical Standard Curve



Precision

Intra assay	n	Mean (E)	CV (%)
#1	24	1.448	1.84
#2	24	1.936	2.44
#3	24	1.185	2.37
Inter assay	**n**	**Mean (IU/ml)**	**CV (%)**
#1	12	13.83	11.93
#2	12	45.28	5.19
#3	12	26.18	12.41

Detection Range

0.45 IU/ml - 100 IU/ml

Sensitivity

1. Analytical Sensitivity

The analytical sensitivity (according to CLSI EP17-A) is defined as the apparent concentration of the analyte that can be distinguished from the zero calibrator. It is 0.45 IU/ml.

2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 100.0% (95% confidence interval: 94.87% - 100.0%).

3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 100.0% (95% confidence interval: 97.72% - 100.0%).

Specificity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of falsepositive results due to cross-reactions.

Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.5 mg/ml bilirubin.

Precautions

1. The test procedure, the information, the precautions and warnings in the instructions for use have to be

strictly followed. The use of the testkits with analyzers 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. Only for research use.
3. All materials of human or animal origin should be regarded and handled as potentially infectious.
4. 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.
5. Do not interchange reagents or Microtiterplates of different production lots.
6. No reagents of other manufacturers should be used along with reagents of this test kit.
7. Do not use reagents after expiry date stated on the label.
8. Use only clean pipette tips, dispensers, and lab ware.
9. Do not interchange screw caps of reagent vials to avoid cross-contamination.
10. Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
11. After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
12. To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
13. The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
14. The TMB Substrate Solution contains NMP. Therefore, the following hazard and precautionary statements apply.

    May damage the unborn child.

    Wear protective gloves/protective clothing.

    IF exposed or concerned: Get medical advice/ attention.
15. Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

Limitations

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