Human Rubella IgG antibody ELISA Kit

Human Rubella IgG antibody ELISA Kit has been designed for the quantification of specific IgG antibodies against Rubella virus in serum and plasma.

Catalog number: ARG82908

Package: 96 wells

TABLE OF CONTENTS

SECTION **Page**

INTRODUCTION 3
PRINCIPLE OF THE ASSAY 4
MATERIALS PROVIDED & STORAGE INFORMATION 5
MATERIALS REQUIRED BUT NOT PROVIDED 5
TECHNICAL HINTS AND PRECAUTIONS 6
SAMPLE COLLECTION & STORAGE INFORMATION 7
REAGENT PREPARATION 7
ASSAY PROCEDURE 8
CALCULATION OF RESULTS 9
INTERPRETATION OF RESULTS 10
QUALITY ASSURANCE 11

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INTRODUCTION

Rubella, also known as German measles or three-day measles, is an infection caused by the rubella virus. This disease is often mild with half of people not realizing that they are infected. A rash may start around two weeks after exposure and last for three days. It usually starts on the face and spreads to the rest of the body. The rash is sometimes itchy and is not as bright as that of measles. Swollen lymph nodes are common and may last a few weeks. A fever, sore throat, and fatigue may also occur. Joint pain is common in adults. Complications may include bleeding problems, testicular swelling, encephalitis, and inflammation of nerves. Infection during early pregnancy may result in a miscarriage or a child born with congenital rubella syndrome (CRS). Symptoms of CRS manifest as problems with the eyes such as cataracts, deafness, as well as affecting the heart and brain. Problems are rare after the 20th week of pregnancy.

Rubella is usually spread from one person to the next through the air via coughs of people who are infected. People are infectious during the week before and after the appearance of the rash. Babies with CRS may spread the virus for more than a year. Only humans are infected. Insects do not spread the disease. Once recovered, people are immune to future infections. Testing is available that can verify immunity. Diagnosis is confirmed by finding the virus in the blood, throat, or urine. Testing the blood for antibodies may also be useful. [Provided by Wikipedia: Rubella]

PRINCIPLE OF THE ASSAY

This assay employs the enzyme immunoassay technique. Specific antigen has been pre-coated onto a microtiter plate. Each sample or Standard A to D are pipetted into the wells and any specific Antibody present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated anti human IgG antibody is added to each well and incubate. After washing away any unbound antibody-enzyme reagent. The immune complex formed by the bound conjugate is visualized by adding TMB substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of specific antibodies in the sample. The color development is stopped by the addition of Stop Solution and the intensity of the color is measured at a wavelength of 450 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate (Rubella Virus antigens)	8 X 12 strips	4°C.
Standard A (0.0 IU/mL); blue cap	2 ml (Ready-to-use)	4°C. The standards are calibrated in accordance with the WHO International Standard; Anti Rubella Immunoglobulin Human; NIBSC code: RUBI-1-94.
Standard B (10 IU/mL); green cap	2 ml (Ready-to-use)	
Standard C (50 IU/mL); yellow cap	2 ml (Ready-to-use)	
Standard D (100 IU/mL); red cap	2 ml (Ready-to-use)	
HRP-Conjugate (Anti-human IgG); black cap	20 ml (Ready-to-use)	4°C
20X Wash buffer	50 ml	4°C
Sample Diluent Buffer	100 ml (Ready-to-use)	4°C
TMB Substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP Solution	15 ml (Ready-to-use)	4°C
Cover foil	1 piece	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450 / 620 nm
- Incubator 37°C
- Vortex / mixer
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times and do not use after the expiry date.
- It is very important to bring all reagents and samples to room temperature (20-25°C) and mix them before starting the test run.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- 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.
- If crystals are observed in the 20X Wash buffer, warm up to 37°C until the crystals are completely dissolved.
- Do not interchange reagents or Microplates of different production lots.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- For further internal quality control each laboratory should additionally use known samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately. The samples can be stored at 2-8 °C up to 5 days or aliquot and store samples at ≤ -20 °C or lower for longer storage. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately. The samples can be stored at 2-8 °C up to 5 days or aliquot and store samples at ≤ -20 °C or lower for longer storage. Avoid repeated freeze-thaw cycles.

Note:

- Heat inactivation of samples is not recommended.
- Before assaying, all samples should be diluted 1+100 with Sample Diluent Buffer. Dispense 10 µL of sample and 1 mL of Sample Diluent Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (E.g., add 50 mL of 20X Wash Buffer into 950 mL of distilled water to a final volume of 1000 mL)

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (20-25°C) before use. Standards and samples should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µL** of **diluted samples**, **Standards** and **controls** into respective wells. Leave one well empty for the **substrate blank**.
3. Cover the plate with the foil and incubate for **60 ± 5 minutes** at **37 ± 1 °C**.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with **1× Wash Buffer (300 µL)** using a squirt bottle, manifold dispenser, or autowasher. The interval between washing and aspiration should be **> 5 sec**. Complete removal of liquid at each time is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

   Note: 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.
5. Add **100 µL** of **HRP-Conjugate** into each well (**except the substrate blank well**). Incubate for **30 minutes** at **RT** in the dark.
6. Wash as according to step 4.
7. Add **100 µL** of **TMB Substrate** to each well (including the well for substrate blank). Cover the plate and incubate for exactly **15 minutes** at **RT** in the dark.

8. Add 100 µL of Stop Solution to each well (including substrate blank wells).
9. Read the OD with a microplate reader at 450 nm within 30 minutes. (620 nm as optional reference wave length) and use the substrate controls as blank.

CALCULATION OF RESULTS

1. Adjust the ELISA Microtiterplate reader to zero using the Substrate Blank. If - due to technical reasons - the ELISA Microplate 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.
2. Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard / control and sample in the plate layout. Measurement using a reference wavelength of 620 nm is recommended. Where applicable calculate the mean absorbance values of all duplicates.
3. In order to obtain quantitative results in IU/mL plot 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 curve (absorbance values on the y-axis, concentrations on the x-axis). Read results from this standard 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.
4. 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

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

INTERPRETATION OF RESULTS

1. < 10 IU/mL (Negative): The sample contains no antibodies against the pathogen.
2. 10-15 IU/mL (Equivocal): 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.
3. >15 IU/mL (Positive): Antibodies against the pathogen are present.

QUALITY ASSURANCE

Limit of detection

Functional sensitivity was determined to be 0.45 IU/ml.

Cross-Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive 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.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 1.84-2.44% and inter-assay precision was 5.19-12.41%.