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Product Information

Rubella Virus IgG ELISA Kit

Catalog Number: EA101084
Storage Temperature: 2 – 8°C

Instruction for Use

THIS KIT IS INTENDED FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

1 INTRODUCTION

Intended Use

The **ORIGENE Rubella Virus IgG Enzyme Immunoassay Kit** provides materials for measurement of IgG-class antibodies to Rubella Virus in human serum and plasma.

2 PRINCIPLE OF THE TEST

The **ORIGENE Rubella Virus IgG ELISA** Kit is a solid phase enzyme-linked immunosorbent assay (ELISA). Microtiter wells as a solid phase are coated with inactivated K1S grade Rubella Virus antigen (strain HPV-77). **Diluted sample** specimens and **ready-for-use controls** are pipetted into these wells. During incubation Rubella Virus-specific antibodies of high specimens and controls are bound to the immobilized antigens. After a washing step to remove unbound sample and control material horseradish peroxidase conjugated anti-human IgG antibodies are dispensed into the wells. During a second incubation this anti-IgG conjugate binds specifically to IgG antibodies resulting in the formation of enzyme-linked immune complexes. After a second washing step to remove unbound conjugate the immune complexes formed (in case of high results) are detected by incubation with TMB substrate and development of a blue color. The blue color turns into yellow by stopping the enzymatic indicator reaction with sulfuric acid. The intensity of this color is directly proportional to the amount of Rubella Virus-specific IgG antibody in the sample specimen. Absorbance at 450 nm is read using an ELISA microtiter plate reader.

3 WARNINGS AND PRECAUTIONS

- For professional use only.
- Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Avoid contact with Stop Solution containing 0.2 mol/L H_2SO_4. It may cause skin irritation and burns.
- TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Allow the reagents to reach room temperature (21 °C – 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the specimen samples will not be affected.
- Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.

4 REAGENTS

Reagents provided

1. ***Microtiterwells***, 12 x 8 (break apart) strips, 96 wells;
   Wells coated with inactivated K1S grade Rubella Virus antigen (strain HPV-77).
   (incl. 1 strip holder and 1 cover foil)
2. ***Sample Diluent ****, 1 vial, 100 mL, ready to use,
   colored green; pH 7.2 ± 0.2.
   Contains anti-human IgG-class antibody.
3. ***Standard* (*Standard 1 - 3*)*,** 3 vials, S1 – S3 with 2.0 mL, ready to use;
   Concentrations: 10, 50, 100 IU/mL,
   colored yellow, white caps.
   The standards are calibrated against WHO International Standard Anti Rubella Immunglobulin, Human (RUBI-1-94)
4. ***Low Control ****, 1 vial, 2.0 mL, ready to use;
   colored yellow, yellow cap.
5. ***High Control****, 1 vial, 2.0 mL, ready to use;
1. colored yellow, red cap.
6. ***Enzyme Conjugate ****, 1 vial, 20 mL, ready to use,
   colored red,
   antibody to human IgG conjugated to horseradish peroxidase.
7. ***Substrate Solution***, 1 vial, 14 mL, ready to use,
   Tetramethylbenzidine (TMB).
8. ***Stop Solution***, 1 vial, 14 mL, ready to use,
   contains 0.2 mol/L H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.
9. ***Wash Solution ****, 1 vial, 30 mL (20X concentrated for 600 mL), pH 6.5 ± 0.1
   see „Preparation of Reagents“.

* Contain non-mercury preservative.

Material required but not provided

- A microtiter plate calibrated reader (450/620 nm ± 10 nm)
- Calibrated variable precision micropipettes
- Incubator 37 °C
- Manual or automatic equipment for rinsing wells
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Timer
- Absorbent paper

Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.

Opened kits retain activity for two months if stored as described above.

Reagent Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.

Wash Solution

Dilute *Wash Solution* **1+19** (e.g. 10 mL + 190 mL) with fresh and germ free redistilled water. This diluted wash solution has a pH value of 7.2 ± 0.2.
Consumption: ~ 5 mL per determination.
Crystals in the solution disappear by warming up to 37 °C in a water bath. Be sure that the crystals are completely dissolved before use.
The diluted Wash Solution is stable for 4 weeks at 2 °C to 8 °C.

Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheets.

Damaged Test Kits

In case of any severe damage to the test kit or components, ORIGENE has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SPECIMEN COLLECTION AND PREPARATION

Serum or plasma can be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.

Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 3 days at 2 °C to 8 °C prior to assaying.
Specimens held for a longer time should be frozen only once at –20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

Specimen Dilution

Prior to assaying dilute each sample specimen **1+100** with *Sample Diluent*;
e.g. 10 µL of specimen + 1 mL of *Sample Diluent* **mix well, let stand for 15 minutes mix well before use.**

For samples with concentrations greater than Standard 3 a second 1:10 dilution of this 1+100 diluted specimen sample should be performed;
e.g. 20 µL of first sample dilution + 180 µL Sample Diluent (mix well).

Please note: Standards and Controls are ready for use and must not be diluted!

6 ASSAY PROCEDURE

General Remarks

- **It is very important to bring all reagents, samples and controls to room temperature before starting the test run!**
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.

- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- To avoid cross-contamination and falsely elevated results pipette specimen samples and dispense conjugate without splashing accurately to the bottom of wells.

During 37°C incubation cover microtiter strips with foil to avoid evaporation.

Test Procedure

Prior to commencing the assay, dilute *Wash Solution*, **prepare specimen samples as described in point 5.3** and establish carefully the **distribution and identification plan** supplied in the kit for all specimens and controls.

Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:

1 well (e.g. A1) for the substrate blank,
1 well (e.g. B1) for the *Low Control,*
3 wells (e.g. from C1 on) for the *Standard 1-3*
1 well (e.g. F1 for the *High Control*

It is left to the user to determine controls and specimen samples in duplicate.

Dispense

100 µL of *Low Control* into well B1
100 µL of *Standard 1* into well C1
100 µL of *Standard 2* into well D1
100 µL of *Standard 3* into well E1
100 µL of *High Control* into well F1 and
100 µL of each d i l u t e d sample with new disposable tips into appropriate wells.
Leave well A1 for substrate blank!

Cover wells with foil supplied in the kit. Incubate for **60 minutes at 37 °C.**

Briskly shake out the contents of the wells.

Rinse the wells **5 times** with diluted *Wash Solution* (**300 µL per well**). Strike the wells sharply on absorbent paper to remove residual droplets.

Important note:

The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

Dispense **100 µL** *Enzyme Conjugate* into each well, **except A1**.

Incubate for **30 minutes at room temperature (20 °C to 25 °C).**

Do not expose to direct sun light!

Briskly shake out the contents of the wells.

Rinse the wells **5 times** with diluted *Wash Solution* (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.

Add **100 µL** of *Substrate Solution* into all wells.

1. Incubate for **exactly 15 minutes at room temperature (20 °C to 25 °C) in the dark**.
2. Stop the enzymatic reaction by adding **100 µL** of *Stop Solution* to each well.
   Any blue color developed during the incubation turns into yellow.
   Note: Very high specimen samples can cause dark precipitates of the chromogen!
3. Read the optical density at **450/620 nm** with a microtiter plate reader **within 30 minutes** after adding the *Stop Solution*.

Measurement

Adjust the ELISA microplate or microstrip reader **to zero** using the **substrate blank in well A1**.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!
Measure the absorbance of all wells **at 450 nm** and record the absorbance values for each control and specimen sample in the distribution and identification plan.
Dual wavelength reading using 620 nm as reference wavelength is recommended.
Where applicable **calculate the mean absorbance values** of all duplicates.

7 CALCULATION OF RESULTS

Calculation of quantitative Results

In order to obtain **quantitative results in IU/mL** plot the (mean) absorbance values of *Low Control* and Standards 1, 2, 3 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 calibration curve (absorbance values on the vertical y-axis, concentrations on the horizontal x-axis).
Read results from this standard curve employing the (mean) absorbance values of each sample specimen and control.
All suitable computer programs available can be used for automated result reading and calculation. The following mathematical functions can be used: 4 PL (4 Parameter Logistics) curve fit, Linear regression or Point to Point calculation of the standard curve. We use ORIGENE regression program for windows (4 parameter Rodbart regression). If other regression software is used, the obtained values have to be validated by the user.

NOTE: Values of additionally (1:10, in total 1:1000) diluted spceimen samples must be multiplied by the appropriate dilution factor in order to obtain correct results! (Dilution: 1:10 = Dilution factor: 10). (See chapter "5.3 Specimen Dilution").

Interpretation of quantitative Results

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

Important notes for the interpretation of results of the ORIGENE Rubella IgG ELISA

If a Rubella infection is suspected during pregnancy it is required to determine the antibody state of the pregnant woman. In case that 65 IU/mL IgG is found in serum and higher, the woman is protected against the infection and there is no risk of an embryonic damage. Results found from 40 – 45 IU/mL are in a grey-zone.

8 QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or ORIGENE directly.

9 LIMITATIONS OF USE

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

Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact ORIGENE.

Liability

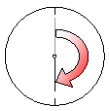
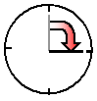
Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

10 REFERENCES

1. Reef Se., Frey Tk., Theall K., Aabernathy E., Burnett Cl., Icenogl J., McCauley Mm., Wharton M. The changing epidemiology of rubella in the 1990s: on the verge of elimination and new challenges for control and prevention. JAMA. 2002 Jan 23-30;287(4):464-72.
2. Mezzasoma L Bacarese-Hamilton T., Di Christina M., Rossi R. Bistoni F., Crisanti A. Antigen microarrays for serodiagnosis of infectious diseases. Clin Chem. 2002 Jan;48(1):121-30.
3. Cooper Lz. Current lessons from 20th century serosurveillance data on rubella. Clin Infect Dis. 2001 Oct 15;33(8):1287.

12 SHORT INSTRUCTIONS FOR USE

	All reagents and specimens must be allowed to come to room temperature (18-25°C) before use.
	Leave well A1 for substrate Blank. Dispense 100 µL of Standards and Control into appropriate wells.
	Dispense 100 µL of sample into selected wells. **(Please note special sample treatment, point 5.3!)**
	Cover wells with foil. Incubate for **60 minutes** at 37 °C
	Briskly shake out the contents of the wells.
	Rinse the wells **5 times** with diluted Wash Solution (300 µL per well).
	Strike the wells sharply on absorbent paper to remove residual droplets.
	Dispense 100 µL of Enzyme-Conjugate into each well.
	Incubate for **30 minutes** at room temperature.
	Briskly shake out the contents of the wells.
	Rinse the wells **5 times** with diluted Wash Solution (300 µL per well).
	Strike the wells sharply on absorbent paper to remove residual droplets.
	Add 100 µL of Substrate Solution to each well.
	Incubate for **15 minutes** at room temperature.

	Stop the reaction by adding 100 µL of Stop Solution to each well.
	Determine the absorbance of each well at 450 nm.

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