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ab247202 Rubella Virus IgG ELISA kit (avidity)

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To indicate the Rubella-specific IgG avidity in human serum or plasma (citrate, heparin) to differentiate between acute and past infection.

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

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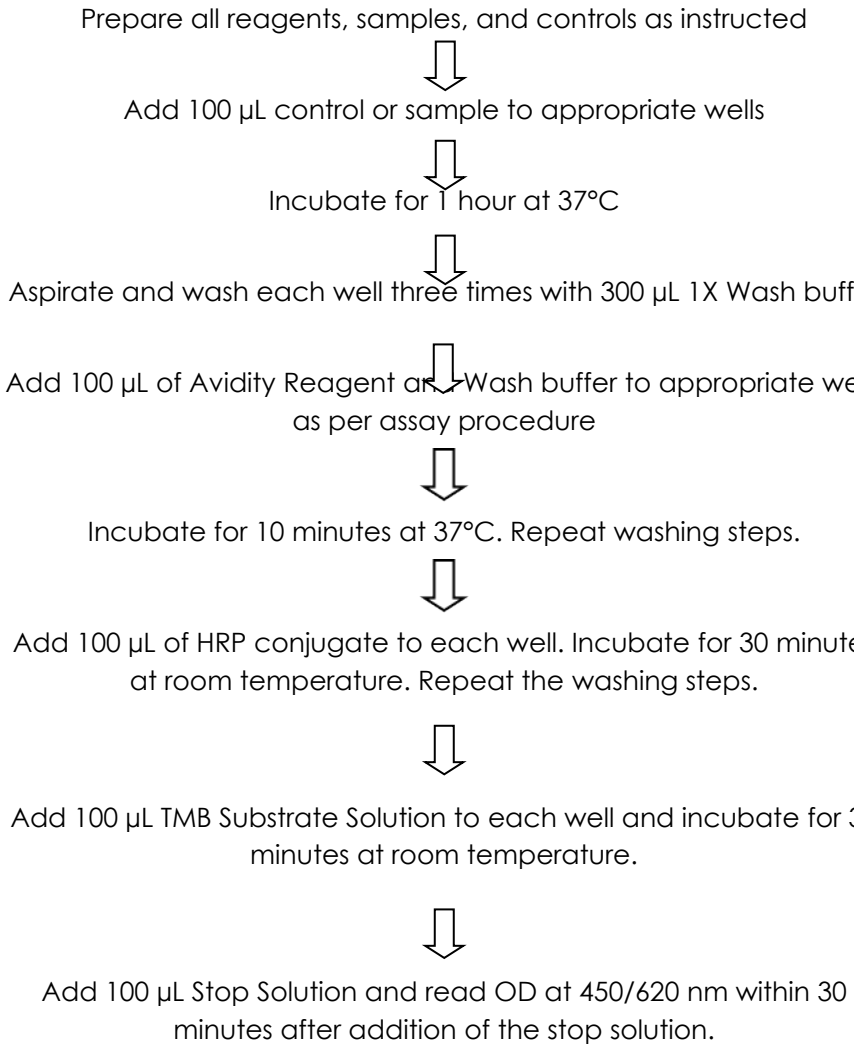
1. Overview

Rubella Virus IgG ELISA kit (avidity) (ab247202) is designed to indicate the Rubella-specific IgG avidity in human serum or plasma (citrate, heparin) to differentiate between acute and past infection.

The qualitative 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 (dual pipetting). After washing the wells to remove all unbound sample material, one well is incubated with avidity reagent and the corresponding well with wash buffer. The avidity reagent removes the low-avidity antibodies from the antigens whereas the high-avidity ones are still bound to the specific antigens. After a second washing step to remove the rest of avidity reagent and low-avidity antibodies, a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a third 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 colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

The presence of IgG antibodies to Rubella Virus indicates the occurrence of the infection but does not distinguish between recent and past infection. Virus-specific IgM antibodies are first detected approximately in ten days and peak at about four weeks post infection. They may persist for several months after acute infections. Based on the evidence that antibody avidity gradually increases after exposure to an immunogen, avidity of IgG antibodies can be used as a marker for distinguishing recent primary from long-term infections. Avidity describes the binding strength of a specific antibody to its antigen. Low-avidity IgG antibodies indicate a primary infection, whereas the presence of IgG antibodies with high avidity points to persistency or reactivation of infection.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Wash Buffer (20X)	50 mL	+4°C
Empty 20 mL bottle for Wash buffer (1X)	1 unit	+4°C
Cover Foil	1 unit	+4°C
Stop Solution	15 mL	+4°C
TMB Substrate Solution	15 mL	+4°C
Rubella Virus Coated Microplate (IgG)	1 unit	+4°C
Rubella Virus anti-IgG Conjugate	20 mL	+4°C
Rubella Virus IgG Standard A (0 IU/mL)	2 mL	+4°C
Rubella Virus IgG Standard B (10 IU/mL)	2 mL	+4°C
Rubella Virus IgG Standard C (50 IU/mL)	2 mL	+4°C
Rubella Virus IgG Standards D (100 IU/mL)	2 mL	+4°C
IgG Sample Diluent	100 mL	+4°C
Avidity Reagent	15 mL	+4°C
IgG high Control	2 mL	+4°C
IgG low Control	2 mL	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Incubator at 37°C
- Microplate reader capable of measuring absorbance at 450/620 nm.
- Deionized water.
- Multi- and single-channel pipettes.
- Plate shaker for all incubation steps.

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 Wash Buffer (20X):

Dilute wash buffer (20X) 1:20 in distilled water (e.g. 10 mL of wash buffer in 190 mL distilled water. The diluted wash buffer is stable for 5 days at room temperature. Warm up the solution to 37°C e.g. in a water bath. Mix well before dilution. Once dissolved, fill 15 mL the supplied empty bottle with 1X Wash Buffer.

9.2 Rubella Virus Coated Microplate (IgG):

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 4 °C.

9.3 Avidity Reagent:

If crystals have formed in the reagent warm up to 37 °C e.g. in a water bath and mix gently until they disappear.

10. Sample Preparation

- Use human serum or plasma (citrate, heparin) Measles Virus IgG positive samples with this assay.
- For samples with antibody concentrations greater than Standard D (100 IU/mL), appropriate higher dilutions should be used.
- If the assay is performed within 5 days after sample collection, the samples should be kept at 4°C; otherwise they should be aliquoted and stored deep-frozen (-70°C). If samples are stored frozen, mix thawed samples well before testing.
- Avoid repeated freezing and thawing.
- Heat inactivation of samples is not recommended.

11. Sample Dilution

- Before assaying, all samples should be diluted 1:100 with IgG Sample Diluent. Dispense 10 µL sample and 990 µL IgG Sample Diluent into tubes to obtain a 1:100 dilution and thoroughly mix with a Vortex.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.
- For avidity determination dual pipetting of standards/controls and diluted samples is needed.

12.1 Prepare all reagents, standards, controls and samples as directed in the previous sections.

12.2 Use the plate layout to plan the location for all standards, controls and samples.

12.3 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

12.4 Add 100 µL of all diluted samples and controls to appropriate wells. Leave well A1/A2 as the Substrate blank. Cover the wells with the cover foil.

12.5 Incubate for 1 hour at 37°C.

12.6 When incubation is completed, remove the foil, aspirate the content of the wells and wash each well 3 x 300 µL with Wash buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be less than 5 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

12.7 Add 100 µL of Avidity Reagent to wells B1, C1, D1, E1 etc., except for the Substrate blank well A1.

12.8 Add 100 µL of Wash buffer to wells B2, C2, D2, E2 etc., except for the Substrate blank well A2.

12.9 Incubate for exactly 10 minutes at 37°C.

12.10 Repeat the washing steps as per 12.6.

12.11 Add 100 µL of HRP conjugate to each well, except A1/A2, and incubate for 30 minutes in the dark at room temperature.

12.12 Repeat the washing steps as per 12.6.

12.13 Add 100 µL of TMB Substrate Solution to each well. Incubate for exactly 15 minutes at room temperature in the dark. A blue color occurs due to an enzymatic reaction.

12.14 Add 100 µL of 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.

12.15 Measure the absorbance at 450/620 nm within 30 minutes after addition of the Stop Solution.

13. Measurement

13.1 Adjust the microplate reader to zero using the Substrate blank.

Δ **Note:** If due to technical reasons that the microplate reader cannot be adjusted to zero using the blank, then subtract its absorbance value from all other absorbance values measured to obtain reliable results.

13.2 Measure the absorbance of all wells at 450 nm and record the absorbance values for each control and sample in the plate layout.

13.3 Biochromatic measurement using a reference wavelength of 620 nm is recommended.

13.4 Where applicable calculate the mean absorbance values for all duplicates.

13.5 Run validation criteria:

- For an assay to be considered valid, the following criteria must be met.
- If these criteria are not met, the test is not valid and must be repeated.

Controls	Absorbance value/Avidity (%)
Substrate Blank	< 0.1
Control low	< 45 %
Control high	> 55%

14. Calculations

– For each patient sample or control calculate the ratio between the absorbance of the well dispensed with Avidity Reagent and the absorbance of the well dispensed with Wash Buffer multiplied by 100:

$$\frac{\text{Absorbance (sample or control) Avidity Reagent}}{\text{Absorbance (sample or control) Wash buffer (1X)}} \times 100 = \text{Avidity (\%)}$$

– For samples with antibody concentrations greater than Standard D (100 IU/mL) appropriate higher dilutions should be used.

Result	Avidity	Interpretation
Low avidity IgG	> 45 %	An avidity index of less than 45 % indicates a primary infection acquired within the past 2 months.
Equivocal	45 – 55 %	No clinical interpretation can be deduced from an equivocal result. It is recommended to take a second sample within an appropriate period of time (e.g. 2 weeks) and repeat testing. If the result of the repeated test is still equivocal, precise statements regarding the time of infection cannot be made.
High avidity IgG	> 55 %	The presence of high-avidity IgG indicates a past infection or reinfection.

Diagnosis of an infectious disease should not be established based on 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.

Antibody Isotypes and State of Infection			
IgG	IgM	IgG-Avidity	Probable result
+	-	low	Vague, further investigation necessary
+	-	high	Indicatives of a past infection
+	+	low	Suggests a current or very recent infection
+	+	high	Suggests a past infection with persisting IgM or reactivation of infection

15. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)