AB Diagnostic Systems

REF **DK.062.01.8** Σ **96** IVD **For *In vitro* Diagnostic Use**

INSTRUCTIONS FOR USE
abia Rubella IgM
Enzyme immunoassay for the detection of IgM antibodies to *Rubella Virus* in human serum or plasma

This Package Insert provides information for Professional Use of the kit.
The kit contains sufficient reagents for 96 (one breakable plate) assays including controls; the kit is intended for manual testing with a possibility of fractional (one strip) use of the kit or for use of the kit on open type automated analyzer for enzyme immunoassay.

I. INTENDED USE

The abia Rubella IgM kit is an enzyme immunoassay for the detection of IgM antibodies to *Rubella virus* in human serum or plasma.

This kit is for diagnostic use by a trained laboratory professional and will not be sold to the general public. All the reagents are for professional in vitro diagnostic use only.

II. INTRODUCTION

Rubella virus is the sole member of the genus *Rubivirus* in the family *Togaviridae*. The disease is transmitted via direct or droplet contact with respiratory secretions. *Rubella virus* multiplies in cells of the respiratory system; this is followed by viremic spread to target organs. Congenital infection is transmitted transplacentally [1].

Infections caused by *Rubella virus* are usually mild and often subclinical. However, serious sequelae for the newborn result from maternal infection. Although rubella vaccination has reduced the incidence of *Rubella virus* infection substantially, it is still important to determine the immune status of women of childbearing age, pregnant women, and health care workers [2].

Humoral and cell-mediated immunity develop following a rubella infection. IgG and IgM antibodies are observed about 14-18 days after rubella infection, at about the time when the rash appears. Rubella IgM antibodies wane quickly and are usually undetectable after 2 months, whereas rubella IgG antibodies persist. In Congenital Rubella Syndrom cases, IgM antibodies are sometimes found for up to 1 year after birth, and persistence of IgG antibodies beyond 6 months of age has been detected in 95% of cases [3].

III. PRINCIPLE OF THE TEST

Scheme of the test procedure is a two-step "sandwich" variant. Microtiter strip wells precoated with the anti-human IgM antibodies. Human IgM antibodies, contained in the test sample, bind with anti-human IgM antibodies. The specific IgM antibodies to *Rubella virus* react with HRP-labeled *Rubella virus*. The presence of bound enzyme indicating the presence in the specimen of specific antibodies is revealed by a color change in the TMB-Substrate. The color development is stopped with the addition of Stopping Reagent, changing the color to yellow. The color intensity of the test sample is directly proportional to the concentration of anti-Rubella IgM.

IV. CONTENT OF THE KIT abia Rubella IgM

4.1. Contents of the reagent kit.

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
Anti-human IgM Coated Strips	Polystyrene stripped 96-well plate (breakable wells) coated with monoclonal antibodies to human immunoglobulin M. Store at 2-8 °C until expiration date.	1 plate
Conjugate	*Rubella virus* antigen labeled with horseradish peroxidase. Transparent or slightly opalescent liquid, yellow colored. Preserving agents: 0.1% ProClin 300; 0.09% phenol. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 11.0 ml
Positive Control, Inactivated	Control sample, containing IgM antibodies to *Rubella virus*. Transparent or slightly opalescent liquid, crimson-red colored. Preserving agents: 0.099% sodium azide, 0.09% phenol. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 1.2 ml
Negative Control, Inactivated	Control sample, negative for antibodies to *Rubella virus*. Transparent or slightly opalescent liquid, green colored. Preserving agent: 0.04% ProClin 300, 0.2% sodium azide. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 2.5 ml
Preliminary Sample Diluent	Sample buffer that is used for preliminary dilution of samples. Transparent or slightly opalescent violet-blue colored liquid. Preserving agents: 0.09% sodium azide. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 11.0 ml
Sample Diluent	Sample buffer that is used to dilute samples before analysis. Transparent or slightly opalescent pale pink colored liquid. Preserving agent: 0.05% ProClin 300. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 11.0 ml
Washing Solution (concentrated 25-fold)	Transparent or slightly opalescent colorless or light yellow liquid, sediment may form that dissolves completely at 35-39 °C and shaking. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 50.0 ml
Stopping Reagent	0.2M sulphuric acid solution. Transparent colorless liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 25.0 ml
TMB-Substrate	Tetramethylbenzidine in citric acid buffer, containing H_2O_2. Transparent colorless liquid. Store at 2-8 °C in a tightly sealed vial.	1 vial 14.0 ml
Plate for preliminary dilution of sera	Polystyrene plate with transparent wells.	1 plate
Protective films for EIA plates		2
Polyethylene bag with a Zip-Lock		1
Disposable plastic dishes for liquid reagents		2
Disposable tips		16

V. PRECAUTIONS

The reliability of the results depends on correct implementation of the following requirements:

- The temperature in the lab should be 18-24 °C.
- Inspect the contents of the box: check the vials and labels integrity. If labels are lost or labels/vials damage, vials should be disposed, and **kit cannot be used**.
- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.
- Carefully reconstitute the reagents avoiding any contamination.
- Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde vapors) or dust that could alter the enzyme activity of the conjugates.
- Use glassware thoroughly washed and rinsed with deionized water or preferably, disposable material.
- Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.
- The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various conjugate or substrate solutions.
- Use a new distribution tip for each sample.
- Well washing is a critical step in this procedure: respect the recommended number of washing cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.
- Never use the same container to distribute conjugate and color development solution.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay's procedure.
- Use high quality water.
- Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

VI. HEALTH AND SAFETY INSTRUCTIONS

- All reagents included in the kit are intended for "*in vitro* diagnostic use".
- Human origin material (inactivated) used in the preparation of Negative Control has been tested and found negative for HBsAg, antibodies to hepatitis C virus, human immunodeficiency virus (HIV-1 and HIV-2), antigen p24 HIV-1.
- Positive Control is prepared from the goat blood (inactivated), contains antibodies to *Rubella virus*.
- Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patients samples as if capable of transmitting infectious disease.
- Do not eat, drink, smoke, or apply cosmetics where immunodiagnostic materials are being handled.

- Any equipment directly in contact with specimens and reagents as well as washing solutions should be considered as contaminated products and treated as such.
- Wear lab coats and disposable gloves when handling reagents and samples and thoroughly wash your hands after handling them.
- Avoid spilling samples or solutions containing samples. Wipe spills immediately and decontaminate affected surfaces.
- Avoid any contact of the TMB-Substrate and the Stopping Reagent with the skin and mucosa.
- Provide adequate ventilation.
- All materials contacted with specimens or reagents, including liquid and solid wastes, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations.

Warning!

Conjugate, Sample Diluent contains ProClin 300.
H317: May cause an allergic skin reaction.
P261: Avoid breathing vapours.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352: IF ON SKIN: Wash with plenty of water.
P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention.

Danger!

Stopping Reagent contains 0.2 M/L sulphuric acid.
H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/ eye protection/face protection.
P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor/ physician.

VII. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED WITH THE KIT:

- Distilled or deionized water.
- Automatic or semiautomatic, adjustable or preset single-channel and multi-channel pipettes with a changeable volume for a set of liquids.
- Disposable pipette tips.
- Microplate incubator at (37.0 ± 1.0) °C.
- Automatic microplate washer.
- Microplate reader equipped with 450 nm or with 450 and 620-680 nm filters.
- Laboratory clock.
- Open type automated analyzer with 450 nm or with 450 and 620-680 nm filters (for automated procedure).

VIII. COLLECTION AND HANDLING OF SPECIMENS

Collection of blood samples should be implemented according to the current practices. Serum, plasma (citrate, heparin, EDTA) may be used. Separate serum or plasma from blood cells as soon as possible to avoid any haemolysis. Extensive haemolysis may affect test performance. Specimens with observable particulate matter should be clarified by centrifugation prior to testing. Suspended fibrin particles or aggregates may yield falsely positive results. The samples after heat inactivation cannot be analyzed.

Samples can be stored at 2-8 °C not more than for 48 hours; they may be deep-frozen at -20 °C. Samples that have been frozen and defrosted more than 1 time cannot be used. Samples with expressed bacterial growing, hemolysis and hyperlipidemia must not be analyzed.

IX. PREPARATION OF THE REAGENTS

1. Ready to use reagents:

- **Anti-human IgM Coated Strips.** Strips are wrapped in a sealed foil-lined bag. Open the bag and remove the tray. Select the number of coated strips required for the assay. Return unused strips in the bag. After the bag has been opened the Coated Strips are stable during the shelf life of the kit at 2-8 °C, provided that the foil-lined bag is resealed with the clip or the foil-lined bag is resealed in Zip-Locked plastic bag. The silica gel bag should not be removed from the foil packaging.
- **Conjugate;**
- **Positive Control;**
- **Negative Control;**
- **Preliminary Sample Diluent;**
- **Sample Diluent;**
- **Stopping Reagent;**
- **TMB-Substrate.**

2. Reagents to prepare:

- **Working Washing Solution**. Thoroughly shake Washing Solution concentrate. To make Working Washing Solution take required amount of concentrate and mix with distilled or deionized water (1:24 ratio) in a separate vial. Thoroughly mix the solution. The prepared Working Washing Solution is stable for 14 days at 18-24 °C or for 28 days at 2-8 °C.

The required volumes of Working Washing Solution for the certain number of strips or plate are tabulated in Table 2.

Table 2

Number of strips to be used		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1 well**
Working Washing Solution	Washing Solution (×25), ml	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	40.0	0.2
	High quality water, ml	72.0	144.0	216.0	288.0	360.0	432.0	504.0	576.0	648.0	720.0	792.0	960.0	4.8

3. Storage of unused reagents

After opening the vials the unused components of the kit: Positive Control, Negative Control, Sample Diluent, Preliminary Sample Diluent, Washing Solution (concentrated 25-fold), Stopping Reagent can be stored in tightly sealed vials until the kit expiration date at 2-8 °C. Coated Strips can be stored until the kit expiration date at 2-8 °C.

Conjugate, TMB-Substrate can be stored in tightly sealed vials within 3 months at 2-8 °C.

X. TEST PROCEDURE

Note: Before use, allow reagents to reach room temperature (18-24 °C) for 30 min.

Step	The assay procedure
1	Add 90 µl of Preliminary Sample Diluent into the wells of the plate for preliminary samples dilution and 10 µl of the tested serum (plasma) samples. Carefully mix by pipetting. Violet-blue color should change to blue-green. If no color change is observed in a well, this indicates the serum has not been dispensed (this does not apply to highly diluted samples).
2	Add 100 µl of Positive and Negative Controls into the wells. 1 strip – Positive Control to 1 well, Negative Control to 2 wells; 2 strip and more – Positive Control to 1 well, Negative Control to 3 wells.
3	Add 90 µl of Sample Diluent and 10 µl of the preliminary diluted samples to the rest of the wells (the final serum dilution ratio is 1:100). Carefully mix fluid in wells by gentle pipetting. Cover the strips with a protective film.
4	Incubate for **30 min** in a microplate incubator at (37.0 ± 1.0) °C.
5	Remove the content of the wells and add into each well 380 µl of Working Washing Solution. Allow a soak time at least 40 seconds and aspirate. Repeat this procedure **4 times**. Incomplete washing will adversely affect assay precision.
6	Add 100 µl of Conjugate to all the wells of the plate. Cover the plate with a protective film.
7	Incubate for **30 min** in a microplate incubator at (37.0 ± 1.0) °C.
8	Remove fluid from wells, wash the plate **4 times** as described in step 5.
9	Add 100 µl of TMB-Substrate into all the wells.
10	Incubate at 18-24 °C for a **20 min** in a dark place.
11	Add 150 µl of Stopping Reagent into wells to stop the reaction results are read by microplate plate reader at wavelength of 450 nm, with reference filter at 620-680 nm. Reading of the absorbance at 450 nm only is possible.

Scheme of the assay is represented in Annex.

Automated analyzer

Validated test protocols and dilution tables of reagent working solutions for different EIA-analyzers can be obtained from the manufacturer upon request (see section XIV). For the instrumentation without established validated protocol follow the section “TEST PROCEDURE” and ensure all requirements described in the section “PRECAUTIONS” are fulfilled. All protocols for automated analyzers must be fully validated before use.

When preparing working reagent solutions for automated EIA procedure, it is necessary to consider "dead" volume of vials and containers used for loading working solutions in the EIA analyzer.

XI. RESULTS

1. Test Validation

For the test to be valid the following criteria must be met. If these criteria are not met the test should be considered invalid and should be repeated.

1. **Positive Control**: the absorbance value should not be less than 1.0;
2. **Negative Control**: the absorbance value should not be more than 0.2.

Calculate cut-off value as:

Cut-Off = average OD value of Negative Control + A (A=0.250)

where **A** – is a coefficient defined by manufacturer during statistical processing for each lot. The coefficient is indicated for each lot in the Instructions for use which is enclosed in the package, and in the Certificate of Analysis.

2. Interpretation of Results

Sample is positive, if the OD value is ≥ Cut-Off.
Sample is negative, if the OD value is < Cut-Off.

XII. PERFORMANCE CHARACTERISTICS

1. Interferences

Hemoglobin (up to 43.24 mg/ml), bilirubin (up to 0.3 mg/ml), lipids (up to 11.4 mg/ml), rheumatoid factor (up to 221 IU/ml) have no influence on the assay results.

2. Cross reactivity

No cross reactivity was found for *Cytomegalovirus* (CMV), *Epstein-Barr* virus (EBV).

3. Diagnostic sensitivity

Diagnostic sensitivity of abia Rubella IgM with 65 anti-Rubella IgM positive samples is 98.4% (95%CI: 91.4-99.7 %).

4. Diagnostic specificity

Diagnostic specificity of abia Rubella IgM with 240 anti-Rubella IgM negative samples is 100% (95% CI: 98.4 -100%).

5. Trueness. Agreement with certified reference measurement procedure

The abia Rubella IgM was compared with the "SERION ELISA classic Rubella IgM", Virion/Serion. 305 serum and plasma samples are tested.

	"SERION ELISA classic Rubella IgM"		
abia Rubella Ab IgM		Positive	Negative
	Positive	61	0
	Negative	4	240

The agreement to comparative assays is 98.7% (301/305) (95%CI: 96.7-99.5%).

6. Precision

The precision of the abia Rubella IgM was determined by 20 days × 3 samples × 2 replicates covering the measuring range.

Intra-assay (within run) precision

Data	Serum sample №1	Serum sample №2	Serum sample №3
Mean (U/ml)	4.50	2.7	1.5
S_r	0.10	0.07	0.06
CV (%)	2.17	2.47	4.12

Inter-assay (between-run) precision

Data	Serum sample №1	Serum sample №2	Serum sample №3
Mean (U/ml)	4.50	2.7	1.5
S_{rr}	0.33	0.19	0.09
CV (%)	7.43	7.33	5.87

XIII. LIMITS OF THE TEST

Negative result indicates that there is no clinically relevant activity of antibodies to the pathogen, but does not exclude the presence of a fresh infection. Reactivity for IgM antibodies to Rubella may indicate current infection, reactivation or recent vaccination.

It is inadmissible to make a diagnosis only on the basis of the anti-Rubella IgM testing results. Results of this test should always be interpreted in conjunction with the patient´s medical history and clinical presentation. Additional testing (detection of a significant rise of IgG antibodies, avidity testing, rubella immunoblot, virus detection or virus isolation) may be needed.

XIV. CONDITIONS OF STORAGE AND TRANSPORTATION

Expiry date is indicated on the packaging. Keep in dark dry place at 2-8 °C.

Transportation should be done at 2-8 °C. Transportation at 9-25 °C is allowed for not more than ten (10) days or at temperature up to 30 °C for not more than seven (7) days. Freezing is prohibited.

AB Diagnostic Systems GmbH
Sportfliegerstraße 4, Berlin, 12487, Germany
Tel. +49 30 208987160, Fax: +49 30 208987199
E-Mail: info@ab-ds.de, www.ab-ds.de

XV. REFERENCES

1. Baron S. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996, Chapter 55.

2. Medici M.C. et al. Evaluation of Rubella Virus Immunoglobulin G (IgG) and IgM Assays with the New Vidia Instrument. JOURNAL OF CLINICAL MICROBIOLOGY, May 2008, Vol. 46, No. 5, p. 1847-1849.

3. Manual for the laboratory diagnosis of measles and rubella virus infection Second edition WHO/IVB/07.01 ORIGINAL: ENGLISH World Health Organization 2007, 11.

4. J.M. Hübschen, et al. Clinical Microbiology and Infection Volume 23, Issue 8, August 2017, Pages 511-515.

5. Sanz J.C. et al. Assessment of RNA amplification by multiplex RT-PCR and IgM detection by indirect and capture ELISAs for the diagnosis of measles and rubella. APMIS. 2010 Mar; 118(3):203-9.

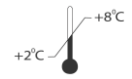
6. Hamkar R. et al. Assessment of IgM enzyme immunoassay and IgG avidity assay for distinguishing between primary and secondary immune response to rubella vaccine. J. Virol Methods. 2005 Dec; 130(1-2):59-65.

7. Tipples G.A., et al., Evaluation of rubella IgM enzyme immunoassays. J. Clin. Virol., Jul 2004, p. 233-238.

8. L. Grangeot-Keros, et al. Evaluation of a new enzyme immunoassay based on recombinant rubella virus-like particles for detection of immunoglobulin M antibodies to Rubella virus. Journal of Clinical Microbiology, Feb. 1997, p. 398-401.

9. Revello M.G. et al. Rubella IgM antibody determination: comparison of two indirect and two capture commercial enzyme immunoassays. Microbiologica. 1987 Oct; 10(4):393-401.

XVI. EXPLANATION OF SYMBOLS

IVD	For in vitro diagnostic use		Storage temperature limitation
	Manufacturer		Expiry date CCYY-MM-DD
	Date of manufacture CCYY-MM		Symbol "exclamation mark"
REF	Catalog number		Symbol "corrosion"
	Sufficient for	**Danger! Warning!**	Signal words
LOT	Batch code		Keep away from sunlight
	Consult Instruction for use		Keep dry
	Top		Fragile, handle with care

Annex

Scheme of the assay

1	**Add**	90 µl of Preliminary Sample Diluent and 10 µl of the samples (conduct on the plate for preliminary dilution of samples)
2	**Add**	100 µl of Positive Control, Negative Control
3	**Add**	90 µl of Sample Diluent
4	**Add**	10 µl of preliminary diluted samples
5	**Incubate**	30 min, (37.0 ± 1.0) °C, microplate incubator
6	**Wash the plate**	Working Washing Solution, not less than 380 µl, 4 times
7	**Add**	100 µl of Conjugate
8	**Incubate**	30 min, (37.0 ± 1.0) °C, microplate incubator
9	**Wash the plate**	Working Washing Solution, not less than 380 µl, 4 times
10	**Add**	100 µl of TMB-Substrate
11	**Incubate**	20 min, 18-24 °C in a dark place
12	**Add**	150 µl of Stopping Reagent
13	**Read the optical density**	450 nm/620-680 nm or 450 nm