

DIA-Rubella-IgM

**Enzyme immunoassay for the detection of
IgM class antibodies against Rubella Virus**

96 tests

Product code: T-12M07C

EXAMPLE FORM

INTENDED USE

Enzyme immunoassay is intended for the detection of IgM class antibodies to Rubella Virus in human serum or plasma by ELISA.

INTRODUCTION

Rubella virus belongs to Togaviridae and is an enveloped RNA virus measuring about 50-70 nm in diameter. The virus causes wide spread disease with ill-defined symptoms characterized as fever, mild constitutional symptoms and generalized rash. But most of case is described as asymptomatic forms that complicates clinical diagnostics of Rubella infection. In childhood this illness does not cause dangerous complications, but if initial infecting occurs during pregnancy there is considerable risks of sever damages of the fetus. In laboratory diagnostics of Rubella virus serological techniques are most widely used. Immunological markers of primary infection is IgM antibodies to Rubella virus, which appear in first days of disease. The maximum level of IgM is detected at 2-3 weeks and decrease in 1-2 months. The specific IgM to Rubella virus antibodies are detected also at vaccination period.

PRINCIPLE OF PROCEDURE

Main components of the kit are an microelisa strips (immunosorbent) and conjugate. Microelisa strips are coated with monoclonal antibodies to human IgM antibodies. The conjugate is presented with antigens of Rubella virus conjugated with horseradish peroxidase.

When investigated specimens of human plasma or serum are placed into wells IgM antibodies to Rubella virus are bound to monoclonal antibodies on the solid phase. Specific IgM antibodies to Rubella virus are detected with the conjugate including Rubella antigens.

After washing non-bound components the developer solution (substrate buffer and chromogen TMB) is added into each well. The peroxidase reaction is stopped by adding the stop-reagent then the absorbance in wells is measured at 450/620 nm.

STORAGE CONDITIONS AND TRANSPORTATION

The kit must be stored and transported at 2-8°C. The kit must not be frozen. Shelf life of the kit is 12 months.

KIT REAGENTS

For *in vitro* diagnostic use.

Each kit contains:

No	Reagents	Presentation
1	Washing solution concentrate Phosphate buffer, containing 3% Tween 20.	2 bottles 2 × 25 ml
2	Microplate 12 strips per plate each with 8 wells coated with monoclonal antibodies to human IgM	1 plate
3	Specimen diluent Phosphate buffer, containing skimmed powdered milk. Preservatives: 0.1% sodium azide, 0.01% benzoic acid.	2 bottles 2 × 30 ml
4	Conjugate diluent Phosphate buffer, containing skimmed powdered milk. Preservatives: 0.05% sodium azide, 0.01% benzoic acid, 0.02% thimerosal.	1 bottle 1 × 15 ml
5	Substrate buffer Citrate-phosphate buffer, containing 0.016% hydrogen peroxide.	1 bottle 1 × 8 ml
6	TMB solution Solution containing 0.03% 3,3',5,5'-tetramethylbenzidine.	1 bottle 1 × 8 ml
7	Positive control Human serum containing IgM class antibodies to Rubella virus. Inactivated by heating. Preservatives: 0.04 % MIT.	1 vial 1 × 0.2 ml
8	Negative control Heating inactivated human serum nonreactive for hepatitis B surface antigen (HBsAg) and antibodies to Rubella virus, HIV ½ and HCV. Preservatives: 0.1% sodium azide.	1 vial 1 × 0.25 ml
9	Conjugate concentrate Rubella antigens conjugated with a horseradish peroxidase (HRP). Preservatives: 0.04% MIT.	1 vial 1 × 1.5 ml
10	Stop-reagent	1 bottle 1 × 15 ml
11	Adhesive film	3 items

Test run is valid if PC is not lower than 0.5 OU.

- Calculate **Cut-off** value.

$$\text{Cut-off} = \overline{NC} + 0.20$$

- Determine the grey zone:

Grey zone is the zone with sample absorbance within the following ranges

$$\text{Cut-off} - 10\% \leq OD \leq \text{Cut-off}.$$

Interpretations

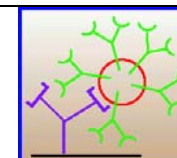
- The sample is considered **negative** if the specimen absorbance is below the grey zone.
- The sample is considered **positive** if the specimen absorbance is greater than the cut-off zone.
- Specimens with absorbance values lying within the grey zone are considered **indeterminate** and should be retested in duplicate. If the repeated results are equivocal, another specimen should be collected for retesting with the original sample.

LIMITATION OF THE PROCEDURE

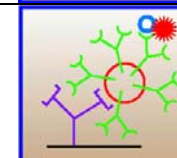
During diagnostics results should be interpreted in conjunction with the patient's clinical condition and the results of others diagnostics procedures.

OVERVIEW OF PROCEDURE

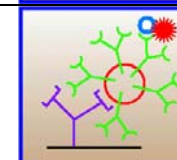
- Dispense 100 µl of diluted investigated specimens and controls in wells
- Incubate for 30 minutes at 37°C
- Wash 4 times with washing solution



- Dispense 100 µl of conjugate solution in wells
- Incubate for 30 minutes at 37°C
- Wash 6 times with washing solution



- Dispense 100 µl TMB substrate in wells
- Incubate for 30 min at room temperature (colouring)
- Stop the reaction by adding 100 µl stop-reagent
- Read the optical density at 450/620 nm



- then fill the wells completely with washing solution (not less than 350 µl per well) avoiding overflow of buffer from one well to another;
- aspirate completely.

Make sure that no fluid remains on the top and the bottom of the strips and strip holder after the last aspiration (e. g. by blotting with absorbent tissue).

* Contact our company for further information on the different types of washers validated by our technical services.

Test procedure

- Fit the strip holder with required number of **strips**.
- Add 100 µl of preliminary diluted specimens (1:51) and controls (1:51) to wells as follows:
 - well A1: 100 µl of the **positive control**.
 - well B1, C1: 100 µl of the **negative control**.
 - well D1, E1, etc.: 100 µl of the **specimens**.
- Cover the plate with adhesive film and incubate at 37°C for 30 minutes.
- Aspirate the contents of all wells and wash the plate with **washing solution** 4 times (according the section *Wash procedure*). If necessary, dry the plate by slight tapping upside-down on absorbent paper.
- Add 100 µl of the **conjugate solution** to each well.
- Cover the plate with adhesive film and incubate at 37°C for 30 minutes.
- Aspirate the contents of all wells and wash the plate with **washing solution** 6 times (according the section *Wash procedure*). If necessary, dry the plate by slight tapping upside-down on absorbent paper.
- Pipette into each well 100 µl of the **TMB substrate**.
- Cover the plate with adhesive film and incubate at 18-25°C for 30 minutes in the dark.
- Add to each well 100 µl of the **stop-reagent** (maintain the same pipetting sequence and rate used for TMB substrate addition).
- Read the absorbance at 450/620 nm using a dual wavelength microplate reader within 5 minutes after stopping the reaction.

An absorbance may be measured at 450 nm (single wavelength) against a blank well; in that case include an empty well in the run.

Interpretation of results

Calculations

NC – absorbance of the negative control

PC – absorbance of the positive control

NC – mean absorbance of the negative control

- Calculate the mean absorbance for negative controls.

Test run is valid if \overline{NC} is not higher than 0.2 optical units (OU).

Exclude any NC, which is higher than 0.2 OU or if it is more than twice exceed the \overline{NC} , and recalculate \overline{NC} of the remaining controls.

ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED

- distilled or deionized water;
- disposable gloves;
- disposable V-shaped troughs;
- vial for reagents preparation (glass or plastic);
- graduated cylinder (1000 ml);
- absorbent paper;
- sodium hypochlorite solution or other accepted disinfectant;
- sodium bicarbonate;
- ethanol, 70°;
- automatic single-channel pipettes (e.g. 5-40, 20-200, 200-1000 µl) with disposable tips;
- automatic multi-channel pipettes (50-300 µl) with disposable tips;
- incubator, 37±1°C;
- microwell wash system*;
- microwell reader* (with dual wavelength 450/620);
- biohazard waste containers for potentially contaminated materials.

* Contact our company for further information on the equipment validated by our technical services.

SAFETY PRECAUTIONS AND WARNINGS

- Use a new tip for pipetting specimens in wells.
- All reagents included in the kit are intended for "in vitro" diagnostic use.
- Wear disposable gloves when handling reagents and samples and thoroughly wash hands after handling them.
- Do not pipette by mouth.
- Human origin material used in the preparation of the negative and positive controls. The positive control has been inactivated by heating. The positive and negative controls were tested and found nonreactive for hepatitis B surface antigen (HBsAg), antibodies HCV and HIV (HIV-1, HIV-2).
- Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.
- Any equipment directly in contact with specimens and reagents as well as the washing solution be considered as contaminated products and treated as such.
- Avoid spilling samples or solution containing samples.
- Spills must be treated with ethyl alcohol 70°. If the contaminating fluid is an acid, spill must be neutralized with sodium bicarbonate and dried with absorbent paper. The materials used for cleaning must be discarded in a contaminated residue container.
- Samples and reagents of human origin, as well as, contaminated material and products must be discarded after decontamination:
 - Either by immersion solid wastes in sodium hypochlorite at a final concentration of 5%, liquid wastes in sodium hypochlorite at a final concentration of 1% during 30 min.
 - Or by autoclaving at 121°C during 2 hours. The best method of inactivating of HIV, HBV, and HCV is an autoclaving.

- DO NOT PLACE SOLUTIONS CONTAINING SODIUM HYPOCHLORITE IN THE AUTOCLAVE.

- Do not forget neutralize acid solutions before autoclave.
- Avoid any contacts substrate solution, chromogen and stop-reagent with skin and mucous covers.
- The negative and positive controls contain sodium azide as a preservative. Sodium azide may react with laboratory plumbing forming copper or lead azides. Such azides are explosive. To prevent azide build-up, flush the pipes with a huge quantity of water if solutions containing azide are disposed of the sink after inactivation.

SPECIMEN PREPARATION

Serum or plasma specimens are to be stored at 2-8°C during 72 hours. If necessary these specimens may be frozen (not more than two freezing-thawing procedures are allowed) at temperature below -20°C.

All specimens containing aggregates and visible suspended particles are to be clarified by centrifugation.

Specimens with sodium azide, hemolysis, hyperlipidemiae or bacterial contamination may not be used in the ELISA procedure.

ASSAY PROCEDURE

Reagents and specimens should be at room temperature (18-25°C) before beginning the assay and can remain at room temperature during testing. Return reagents to 2-8°C after use.

Reagents preparation (for 8 wells)

Microplate

Open the bag and remove the strip plate. Return unused strips in the pack. Reseal the pack and store at 2-8°C.

The strips are stable for 4 weeks at 2-8°C after opening the pack.

Washing solution

Check **Washing solution concentrate** for the presence of salt crystals. If crystals are seen in the solution, dissolve them by heating at 35-37°C.

Dilute the 4 ml of the **Washing solution concentrate** with 120 ml of the distilled or deionised water, shake intensively to dissolve any crystals that may be present.

Washing solution is stable for 10 days at 2-8°C.

Serum dilution (specimens and controls)

Investigated serum samples, positive and negative controls are preliminarily diluted (1:51) with the **Specimen diluent**. For example, 10 µl of serum is added to 500 µl of the **Specimen diluent**. Dilution of specimens should be performed before use. *Carefully repipette the mixture in wells.*

Note: Dilution of serum and controls is not to be straight performed in wells of the strip plate. It can be run in separate vessels.

Conjugate solution

Dilute 100 µl of the **Conjugate concentrate** with 1 ml of the **Conjugate diluent** (1:11) in a clean vial. Mix well avoiding foaming.

Conjugate solution has to be prepared before use.

Conjugate solution is stable for 6 hours at 2-8°C.

TMB substrate

In order to prepare **TMB substrate**, dilute 0.5 ml of the **TMB solution** with 0.5 ml of the **Substrate buffer** in a clean vial (1:1). Mix well. TMB substrate must be colourless before use.

The TMB substrate must be kept away from light and no solutions contact with metals or metal ions is allowed.

The solution has to be prepared before use.

TMB substrate is stable for 2 weeks at room temperature (18-25°C) if kept in the dark.

Procedural notes

Authenticity of results depends on correct execution following instructions:

- Reagents should not be used beyond the expiry date shown on the package label.
- Reagents should not be mixed from different lots during performing test.
- Reagents and samples should be at room temperature (18-25°C) before testing begins. Return the reagents to 2-8°C after use.
- The temperature in room where performing analysis should be in the range 18-25°C.
- It should accurately dissolve reagents avoiding its contamination.
- Do not perform the test in the presence of reactivity vapours (for example, from sodium hypochlorite, acids, alkalis, or aldehydes) or dust because the enzymatic activity of the conjugate may be affected.
- Use glass vessels thoroughly washed and rinsed with deionized water or use disposable ones.
- Do not allow drying contents of wells on all stages of procedure.
- Enzyme reaction is sensitive to metal ions, so avoid contacting with metal elements.
- TMB substrate (solution for chromogen preparation + chromogen TMB) is to be colourless. Appearance of colouring after dilution is evidence of unavailability for using and solution is to be replaced. The solution is to be prepared in clean plastic ware or clean glassware. The reagent is to be kept in dark.
- Prevent the direct light to fall on the working surface during ELISA procedure.
- Use a new tip for brining specimens in wells.
- Never use the same trough for distribution conjugate and TMB substrate.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay procedure.

Wash procedure

Washing must be performed strictly according to the instructions, as insufficient plate washing leads to incorrect results.

Use automatic washer*, as recommended; in case of its absence or faulty work – use multi-channel pipette for washing.

Follow this procedure in each washing:

- aspirate the wells contents completely into a waste flask;