Rubella IgM GENLISA® ELISA

REF : KDT010

Ver 1.4

IVD

Enzyme Immunoassay for the Qualitative Determination of Rubella Virus IgM in human serum and plasma.

IVD	**For In-vitro Diagnostic Use**	**REF**	**Catalog Number**
	Store At	**LOT**	**Batch Code**
	Manufactured By		**Biological Risk**
	Expiry Date		**Consult Operating Instructions**

REF KDT010 Σ 96 tests

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Introduction:

Rubella is caused by rubella virus (RV), belongs to the genus Rubivirus in the family Togaviridae . The virion is enveloped by a lipid membrane and possesses a positive-sense single-stranded RNA and usually represents as a mild illness characterized by low-grade fever, a short-lived morbilliform rash, and lymphadenopathy. The infection can be transmitted to pregnant women early during pregnancy and may result in miscarriage, stillbirth, or infants born with birth defects known as congenital rubella syndrome (CRS).

Intended Use:

The Rubella IgM GENLISA® ELISA is intended for the qualitative determination of Rubella Virus IgM class antibodies in human serum and plasma.

Principle:

Rubella IgM GENLISA® ELISA method employs enzyme linked immunosorbent assay (ELISA) technique. Purified anti-human IgM monoclonal antibodies are pre-coated onto microwells. Samples and Controls are pipetted into microwells and Rubella IgM antibody present in the sample is bound by the antibodies. And then enzyme labelled antigen is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the ready to use TMB substrate solution is added to microwells and color develops proportionally to the amount of Rubella IgM antibody present in sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Microtiter Coated Plate (8x12 wells) - 1 no
2. Negative Control - 1 ml
3. Positive Control - 1 ml
4. Enzyme Conjugate - 6.5 ml
5. (20X) Wash Buffer - 25 ml
6. Sample Diluent - 11 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Instruction Manual.

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Timer.
7. Absorbent Paper.

Handling/Storage:

1. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.

2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.

Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.

Reagent Preparation:

1. Wash Buffer (1X) Dilution: To make Wash Buffer (1X), add 25ml of Wash Buffer (20X) to 475ml of DI water. This is the working solution.
2. Allow all components to reach RT (Room Temperature) prior to use in the assay.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. Add **100 ul Sample Diluent** to the sample wells.
3. Add **10 ul Sample** to the respective sample wells. Mix gently.
4. Dispense **100 ul Negative Control** and **100 ul Positive Control** to the negative and positive wells respectively.
5. Shake gently for 30 seconds to mix well. Incubate at 37°C for 20 minutes.
6. Aspirate and wash plate 5 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
7. Add **50 ul** of **Enzyme Conjugate** to each well except the blank well.
8. Incubate at 37°C for 20 minutes.
9. Repeat the Aspiration / Wash Step.
10. Add **100 ul** of **TMB Substrate** into each well except blank well.
11. Incubate at 37°C for 10 minutes.
12. Add **100 ul** of **Stop Solution** into each well except blank well. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

Interpretation of Results:

Determine the Mean Absorbance for each set of duplicate Controls and Samples. Results are interpreted qualitatively by calculating a cut-off value for each sample on the basis of the cut-off determined. Read Absorbance at 450nm with an ELISA reader.

Cut-Off value (CO) = OD_{mean} of Negative Control x 2.1

Note: incase the ODmean of Negative Control is < 0.05 then assume the same as 0.05
Calculated by actual value when mean negative control OD Value is > 0.05.

Positive Results: **OD value ≥ CO**
Specimens giving an absorbance equal to or greater than the CO are considered initially reactive, which indicates that Rubella IgM has probably been detected using Rubella IgM ELISA. All initially reactive specimens should be retested in duplicates using Rubella IgM ELISA before the final assay results interpretation. Repeatedly reactive specimens may be considered positive for Rubella IgM with Rubella IgM ELISA.

Negative Results: **OD value < CO**
Specimens giving absorbance less than the CO are negative for the assay, which indicates that no Rubella IgM has been detected with the Rubella IgM ELISA.

Cut Off Value	OD_{mean} of Negative Control x 2.1
Positive	>= CO
Negative	< CO

Validity of the test:

The test is valid if the following conditions are met,

Mean Absorbance of Negative Control ≤ 0.1
Mean Absorbance of Positive Control ≥ 0.8

Limitations of Method:

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

Performance Characteristics:

Sensitivity:
Limit of Detection: When detecting anti-rubella virus antibody limits, laboratory quality control positive samples diluted till 1:8 with the Rubella IgM ELISA kit should be in the positive.

Specificity:
The recombinant antigen used in the kit is specific for Rubella Virus.

Precision:
Intra-Assay: CV% ≤15%.
Inter-Assay: CV% ≤20%

Safety Precautions:

- **This kit is For In-vitro Diagnostic Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed.

- Do not smoke, eat or drink while handling kit material.
- Always use protective gloves.
- Never pipette material by mouth.

- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

Krishgen Pudgala LLP does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Pudgala LLP, or against damages resulting from such non-Krishgen Pudgala LLP made products or components. Krishgen Pudgala LLP passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Pudgala LLP.

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Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add **100 ul Sample Diluent** to the **respective sample wells**.
3	Add **10 ul Sample** to the **respective sample wells**. Mix gently.
4	Dispense **100 ul Negative Control** and **100 ul Positive Control** to the negative and positive well respectively.
5	Shake gently for 30 seconds to mix well. **Incubate** at **37ºC** for **20 minutes**.
6	Aspirate and **wash plate 5 times** with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
7	Add **50 ul** of **Enzyme Conjugate** to each well except the blank well.
8	Seal the plate and **incubate** at **37ºC** for **20 minutes**.
9	Repeat the Aspiration / Wash Step.
10	Add **100 ul** of **TMB Substrate** into each well except blank well.
11	**Incubate** at **37ºC** for **10 minutes**.
12	Add **100 ul** of **Stop Solution** into each well except blank well. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

SYMBOLS KEY

MTP	**Microtiter Plate (8x12 wells)**
PC	**Positive Control**
NC	**Negative Control**
CONJ	**Enzyme Conjugate**
SAMP DIL	**Sample Diluent**
20X WASH BUF	**(20X) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**