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RD-Ratio Diagnostics GmbH

Instructions for use

Rubella IgM ELISA

REF ID E-1500

96

+2 +8 °C

IVD

CE 0483

RD-Ratio Diagnostics Germany

Rubella IgM ELISA (µ-capture)

1. Intended use and principle of the test

Enzyme Immunoassay for the determination of Rubella IgM antibodies in human serum.

The test for the assay of Rubella IgM ELISA is based on the principle of the capture of these immunoglobulins and subsequent identification of those, which are specific, making use of their ability to bind an antigen conjugated to peroxidase. The capture is performed using monoclonal antibodies bound to the solid phase (microtitration strips). The antigen is composed of purified and inactivated Rubellavirus antigen.

2. Advice on handling the test

2.1 Reliability of the test results

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions.
It is recommended that each laboratory establishes its own reference intervals. The values reported in this test instruction are only indicative.
The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.

2.2 Complaints

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

2.3 Warranty

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

2.4 Disposal

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.
The appropriate safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the control: ISO 11014-1.

2.5 Interference

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

2.6 Precautions

For *in vitro* use only.
The following universal Good Laboratory Practices should be observed:

Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose of all reagents and materials in compliance with applicable regulations.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

This kit may contain some reagents made with human source material (e.g. serum or plasma) or used in conjunction with human source materials. The material in this kit has been tested by CE marked methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HBsAg. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 4th Edition, April 1999.

WARNING AND PRECAUTION:

Some of the reagents in this kit contain sodium azide as a preservative at concentrations below the regulatory limit of < 0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes, and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system.

3. Storage and stability

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Do not mix various lots of any kit component within an individual assay.

4.1 Contents of the kit

ID E-0031	µ IGM	**Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, coated with Anti-human IgM Antibody
BA E-0030	WASH-CONC 50x	**Wash Concentrate**	1 x 20 mL	concentrate, dilute content with dist. water to a final volume of 1000 mL Storage: up to 6 months 2-8 °C
ID E-1540	CONJUGATE	**Conjugate**	1 x 12 mL	ready for use, anti Rubella virus antigen labelled with peroxidase, in a phosphate buffer solution with 0.02% Proclin
BA E-0055	SUBSTRATE	**Substrate**	1 x 12 mL	ready for use, containing a solution of tetramethylbenzidine (TMB)
BA E-0080	STOP-SOLN	**Stop Solution**	1 x 12 mL	ready for use, containing 0.25 M H_2SO_4
ID E-0060	DILUENT	**Diluent**	2 x 50 mL	ready for use, containing a protein solution with 0.09% sodium azide as a preservative
ID E-1551	CONTROL -	**Negative Control**	1 x 2 mL	ready for use
ID E-1552	CONTROL ±	**Cut-off Control**	1 x 2 mL	ready for use
ID E-1553	CONTROL +	**Positive Control**	1 x 2 mL	ready for use

4. 2 Additional materials and equipment required but not provided in the kit

- Microtitration plate reader capable of absorbance measurement at 450 nm
- Deionized/Distilled water
- Precision pipette to deliver 10 µL, 100 µL, and 1 mL
- Semi-automatic pipette to deliver 100 µL
- Automatic microtitration plate washer
- Absorbent materials for blotting the strips
- Temperature controlled water bath (37°C) or similar heating device

5. Sample collection and storage

Serum should be used, and the usual precautions for venipuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at –20°C. Do not use hemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

6. Test procedure

PREPARATION FOR ASSAY

A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert.

- Bring all kit reagents and specimens to room temperature (~25°C) before use and thoroughly mix the reagents and samples before use by gentle inversion.
- Do not mix various lots of any kit component within an individual assay.
- Do not use any component beyond the expiration date shown on its label.
- Incomplete washing will adversely affect the outcome and assay precision.
- To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed to add the TMB Chromogen Solution.
- Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination of the TMB Chromogen Solution with the Conjugate. Use a clean disposable pipette tip for each reagent. Avoid pipettes with metal parts.
- Containers and semi-automatic pipette tips used for the Conjugate and TMB can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage.
- The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies.
- Use high quality water.
- Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

6.1 Preparation of reagents

Washing Solution

Dilute the 20 mL Wash Concentrate with distilled water to a final volume of 1000 mL.
Storage: up to 6 months at 2–8°C.

Microtitration Strips

Remove the support and strips to be used from the foil package, and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.

6.2 Assay Procedure

Allow all specimen and reagents to reach room temperature (20-25°C). Serum samples and controls should be assayed in duplicate.

1.	Dilute serum samples **1:101** distributing **10 µL** of serum into **1 mL** of **Diluent**.
2.	Pipette **100 µL** of each **diluted serum sample** and ready to use **controls** to the appropriate wells. Leave one well for the blank, performed using 100 µL of the TMB-substrate at the substrate incubation step.
3.	**Incubate** for **45 minutes** at **37°C**. Aspirate and **wash each well four (4) times** for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material. *Use of an automatic microplate washer is recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 300 uL of the Wash Solution into each well, and (c) repeat step (a) and (b) four times.*
4.	Add **100 µL** of **Conjugate** into each well.
5.	**Incubate** for **45 minutes** at **37°C**. Aspirate and wash each well four times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
6.	Add **100 µL** of **Substrate** to each well using a dispenser.
7.	**Incubate** for **15 minutes** at **RT** (20-25°C). Avoid exposure to direct sunlight.
8.	Add **100 µL** of **Stop Solution** to each well using a dispenser.
9.	**Read the absorbance** of the solution in the wells within 30 minutes, using a microplate reader set to **450 nm**. If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 600 or 620 nm.

7. Calculation of results

Calculate the mean absorbance for each control and unknown.

Qualitative results:

If the absorbance of the sample is higher than that of the Cut-Off, the sample is positive for the presence of specific IgM.
Calculate the ratio between the average OD value of the sample and that of the Cut-Off. The sample is considered:

Positive: if the ratio is > 1.1.

Doubtful: if +/- 10% of the Cut-Off.

Negative: if the ratio is < 0.9.

If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

7.1 Limitations of the procedure

- A serum sample obtained during the early phase of infection, when only IgG antibodies are present, may be negative by this procedure.
- The test result should be used in conjunction with information available from the evaluation of other clinical and diagnostic procedures.
- Avoid repeated freezing and thawing of reagents and specimens.
- Grossly hemolyzed, icteric or lipemic specimens should be avoided.
- Heat inactivated sera should be avoided.

7.2 Quality control

Subtract the value of the blank from all the other readings. The OD values of the Cut-Off Control must be at least 0.2. The Positive Control must have an OD at least 1.5 times that of Cut-off control.

9. Assay characteristics

Sensitivity and Specificity

101 human sera were analyzed by this Rubella virus IgM ELISA and a commercial ELISA as reference method. Out of 101 samples, 11 were positive for the presence of IgM antibodies to Rubella virus by this ELISA and commercial ELISA showed 11 of them as positive. The results are summarized below.

	Positive	Negative
This ELISA	11	90
Commercial ELISA	11	90

Precision

Inter-assay			
No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean	1.93	0.45	0.12
SD	0.08	0.03	0.01
CV%	**4.06**	**6.66**	**8.33**

Intra-assay			
No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean	2.15	0,44	0.10
SD	0.25	0.02	0.01
CV%	**11.62**	**0.54**	**10.00**

Interference study

Interferences with lipemic, hemolytic or icteric sera are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!