

Distributed by :

DIAsource ImmunoAssays SA	Phone: + 32 67 88 99 99
Rue de l'Industrie 8	Fax: + 32 67 88 99 90
1400 Nivelles	Email : tech.support@diasource.be
Belgium	Website : www.diasource.be

INTENDED USE

The KAPRRVG14 Rubella IgG Elisa test system, distributed by DIAsource, is an Enzyme-Linked Immunosorbent Assay kit providing material for the detection of IgG-class antibodies to Rubella virus in human serum in order to determine the immune status of the patient and / or latent Rubella infection. This assay is intended For In Vitro Use Only.

SUMMARY AND EXPLANATION

The Rubella virus, most commonly known as the German or 3-day measles is an RNA virus that belongs to the family Togaviridae and contains three major structural proteins, E1, E2 and C. It is spread through direct or droplet contact from nasopharyngeal secretions. The infection is highly contagious and affects children, 5-14 years as well as young adults. Rubella infection, however, is largely benign with symptoms ranging from subclinical to a disease characterized by an erythematous rash, low-grade fever, headache, lymphadenopathy, arthralgia, and conjunctivitis. Immunizations and natural infection both confer lifelong immunity and reinfection is extremely rare. Congenital Rubella infection, unlike acquired infection, may cause disastrous clinical effects to the unborn child. A fetus may be stillborn or have such abnormalities as bone and cardiovascular defects, mental retardation, encephalitis, hepatomegaly, splenomegaly, thrombocytopenic purpura, cataracts and microcephaly. Because of severity of the complication from infection, detection in pregnant women is paramount. Therefore, it is important that the level of immunity be determined in women of child-bearing age, pregnant women, neonates who were exposed in utero, and others who may have been in close contact. The clinical recognition of Rubella infection is highly unreliable, and subclinical cases are frequent. Serological testing has been shown to be an effective method of detecting infection.

PRINCIPLE OF THE TEST

The KAPRRVG14 Rubella IgG kit is based on the ELISA technique. In the assay, calibrators and unknowns are incubated in microtitration wells coated with purified and inactivated Rubella virus antigen. After incubation and washing, the wells are treated with the conjugate, composed of anti-human IgG antibodies labeled with peroxidase. After a second incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by wavelength absorbance measurement at 450 nm. The absorbance measured is directly proportional to the concentration of anti-Rubella virus IgG antibodies present.

REAGENTS

The Rubella virus IgG ELISA kit, distributed by DIAsource, contains sufficient reagents for 96 wells. Each kit contains the following reagents:

MATERIAL PROVIDED

Rubella Antigen-Coated Microtitration Strip



Quantity : 1 plate

Wash Concentrate

WASH	SOLN	CONC
------	------	------

Quantity : 1 bottle

Sample Diluent

DIL	SPE
-----	-----

Quantity : 1 bottle

TMB-Substrate

CHROM	TMB
-------	-----

Quantity : 1 bottle

Calibrator 0

CAL	0
-----	---

Quantity : 1 vial

Calibrator 1

CAL	1
-----	---

Quantity : 1 vial

Calibrator 2

CAL	2
-----	---

Quantity : 1 vial

Calibrator 3

CAL	3
-----	---

Quantity : 1 vial

Calibrator 4

CAL	4
-----	---

Quantity : 1 vial

2nd Antibody Conjugate

Ab	HRP
----	-----

Quantity : 1 bottle

Stopping Solution

STOP	SOLN
------	------

Quantity : 1 bottle

MATERIAL NOT PROVIDED

- 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 material for blotting the strips
- Incubator capable of maintaining a temperature of 37 +/- 2°C

Antigen-Coated Microtitration Strips

One strip holder containing 12x8 (96) microtitration wells coated with purified inactivated *Rubella virus* antigen. Store at 2-8°C until expiration date. 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.

Wash Concentrate

One bottle, 100 ml, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% Brig 35 per weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.

Sample Diluent

One bottle, 100 ml, containing BSA solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

Rubella IgG Calibrators

Five vials, each 2 ml of human serum in a 0.01 M phosphate buffer containing BSA with 0.09% sodium azide as a preservative. The values for Calibrator 1 represents the Cut-Off control. Store at 2-8°C until expiration date.

2nd Antibody Conjugate

One bottle, 12 ml, containing anti-human IgG monoclonal antibodies labeled with peroxidase, in a phosphate buffer solution with 0.02% Proclin. Store at 2-8°C until expiration date.

TMB-Substrate

One bottle, 12 ml, containing tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer, pH 3.8. Store at 2-8°C until expiration date.

Stopping Solution

One bottle, 15 ml, containing 0.3 M H₂SO₄ in solution. Store at 2-8°C until expiration date.

PRECAUTIONS

For In Vitro Use

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 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.

For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

SPECIMEN COLLECTION AND HANDLING

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.

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. 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.

PREPARATION OF REAGENTS:

Wash Solution

Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, they should be dissolved at 37°C before dilution. Pour 100 ml of the Wash Concentrate into a clean container and dilute by adding 900 ml of deionized/distilled water. Mix thoroughly by inversion. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.

Microtitration Strips

Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

Assay Procedure

All specimens and reagents to reach room temperature (~25°C) before use. Serum Samples and Ready-to-use-Calibrators should be assayed in duplicate. Cut-Off Serum (Calibrator 1) should be assayed in triplicate.

1. Mark the microtitration strips to be used.
2. Dilute serum samples 1:101 distributing 10 µl of serum into 1 ml of Sample Diluent.
3. Pipette 100 µl of each diluted serum sample Calibrators to the appropriate wells. Leave one well for the substrate blank.
4. Incubate for 45 minutes at 37°C.
5. 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.
NOTE: Use of an automatic microplate washer is strongly 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 0.35 ml of the Wash Solution into each well, and (c) repeat step (a) and (b) four times.
6. Add 100 µl of Enzyme-Labeled 2nd Antibody into each well.
7. Incubate for 45 minutes at 37°C.
8. 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.
9. Add 100 µl of TMB Chromogen Solution to each well using a dispenser.
10. Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
11. Add 100 µl of Stopping Solution to each well using a dispenser.
12. 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.

RESULTS

Calculate the mean absorbance for each calibrator and unknown.

Qualitative results:

The Cut-off control corresponds to Calibrator 1.

If the absorbance of the sample is higher than that of the Cut-Off, the sample is positive for the presence of specific IgG.

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 $\pm 10\%$ 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.

Semi-quantitative results:

The anti-*Rubellavirus* IgG concentration of each sample can be expressed in International Units/ml (IU/mL) according to PEI, Germany. A graph can be constructed by plotting the IU/mL against the average OD of the controls; when the OD of the sample is reported on the graph, the IU/mL contained in the serum sample can be calculated. A standard curve must be performed for each run.

Positive/Negative results can be expressed in IU as follows:

Positive : sample concentration > 11 IU/mL

Negative: sample concentration < 9 IU/mL

Equivocal: sample concentration ranges between 9 and 11 IU/mL.

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

LIMITATIONS OF THE PROCEDURE

- A serum sample obtained during the acute phase of infection, when only IgM 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.
- Serological data of immunocompromised patients and newborn children have restricted value.

QUALITY CONTROL

Subtract the value of the blank from all the other readings. The OD values of Calibrator 1 must be at least 0.2. Calibrator 4 must have an OD at least 3 times that of Calibrator 1.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

150 well selected human sera, collected from a clinical laboratory in Frankfurt/Germany, were analyzed by this Rubella IgG Elisa and a reference Elisa method. Out of 150 samples, 103 were positive for the presence of IgG antibodies to Rubella virus by DIAsource Elisa, and reference method also showed 103 of them as positive. The DIAsource Kit KAPRRVG14 has 100% sensitivity and 100% specificity. An analytical comparison between two assays showed $R^2=0.86$ which is acceptable considered a serological assay. The results are briefly summarized below (see Tab. 1).

Tab.1.

Assay Comparison	DIAsource		
TEST A	Positive	Negative	Brd. Line
Positive	103	0	0
Negative	0	47	0
Brd Line	0	0	0

2. Precision

2. Inter-assay Study				3. Intra-assay study			
No of Replicates	Serum	Serum	Serum	No of Replicates	Serum	Serum	Serum
32	1	2	3	80	1	2	3
Mean	0.03	0.6	0.98	Mean	0.038	0.44	1.19
SD	0.003	0.01	0.02	SD	0.004	0.02	0.037
CV%	9.9	2.1	2.1	CV%	11.2	5.3	3.1

3. Interferences

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

4. Analytical Sensitivity

The DIAsource Rubella IgG ELISA has an analytical sensitivity up to 2.3 IU/mL.

REFERENCES

1. G.B. Wisdom: Enzyme-Immunoassay. Clin. Chem. 22: 1243 (1976).
2. L. Schaefer, J. Dyke et al. Evaluation of microparticle enzyme immunoassays for immunoglobulins G and M to Rubella virus and Toxoplasma gondii on the Abbott IMx automated analyzer. J. Clin. Microbiol. 27: 2410 (1989).
3. Chaye, HH, et al: Cellular and humoral immune responses to Rubella virus structural proteins E1, E2, and C. J. Clin Microbiology, Washington, DC, 1992, pp. 596-599.
4. Mahony, JB, and Chernesky, MA: Rubella virus. In Rose, NR, et al (eds): Manual of Laboratory Immunology, ed. 4. ASM, Washington, DC, 1992, pp. 600-605.



RD-RatioDiagnostics
61348 Bad Homburg
Urseler Str. 26
Germany

Revision date : 2010-11-30

	<u>Used symbols</u>
	Consult instructions for use
	Storage temperature
	Use by
LOT	Batch code
REF	Catalogue number
CONTROL	Control
IVD	In vitro diagnostic medical device
	Manufacturer
	Contains sufficient for <n> tests
WASH SOLN CONC	Wash solution concentrated
CAL 0	Zero calibrator
CAL N	Calibrator #
CONTROL N	Control #
Ag 1251	Tracer
Ab 1251	Tracer
Ag 1251 CONC	Tracer concentrated
Ab 1251 CONC	Tracer concentrated
	Tubes
INC BUF	Incubation buffer
ACETONITRILE	Acetonitrile
SERUM	Serum
DIL SPE	Specimen diluent
DIL BUF	Dilution buffer
ANTISERUM	Antiserum
IMMUNOADSORBENT	Immunoabsorbent
DIL CAL	Calibrator diluent
REC SOLN	Reconstitution solution
PEG	Polyethylene glycol
EXTR SOLN	Extraction solution
ELU SOLN	Elution solution
GEL	Bond Elut Silica cartridges
PRE SOLN	Pre-treatment solution
NEUTR SOLN	Neutralization solution
TRACEUR BUF	Tracer buffer
	Microtiterplate
Ab HRP	HRP Conjugate
Ag HRP	HRP Conjugate
Ab HRP CONC	HRP Conjugate concentrate
Ag HRP CONC	HRP Conjugate concentrate
CONJ BUF	Conjugate buffer
CHROM TMB CONC	Chromogenic TMB concentrate
CHROM TMB	Chromogenic TMB solution
SUB BUF	Substrate buffer
STOP SOLN	Stop solution
INC SER	Incubation serum
BUF	Buffer
Ab AP	AP Conjugate
SUB PNPP	Substrate PNPP
BIOT CONJ CONC	Biotin conjugate concentrate
AVID HRP CONC	Avidine HRP concentrate
ASS BUF	Assay buffer
Ab BIOT	Biotin conjugate
Ab	Specific Antibody
SAV HRP CONC	Streptavidin HRP concentrate
NSB	Non-specific binding
2nd Ab	2nd Antibody
ACID BUF	Acidification Buffer
DIST	Distributor