SERION ELISA *classic* Rubella Virus IgG/IgM

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SERION ELISA *classic* Rubella Virus IgG/IgM

Enzyme Immunoassay for detection of human antibodies (IgG/IgM)

For Sale in the U.S. for Research Use Only. Not for use in diagnostic procedures.

SERION ELISA *classic* Rubella Virus IgG	Order Nr.: ESR129G
SERION ELISA *classic* Rubella Virus IgM	Order Nr.: ESR129M

1 INTENDED USE

The SERION ELISA *classic* Rubella Virus IgG and IgM tests are quantitative and qualitative immunoassays for the detection of human antibodies directed against Rubella Virus in serum or plasma. Furthermore, the SERION ELISA *classic* Rubella Virus IgG test allows for determination of IgG antibody avidity by using the corresponding avidity reagent.

2 BACKGROUND

Rubella Virus is a human pathogen belonging to the Togavirus family of RNA viruses. It is transmitted by droplets and direct contact. Infection is characterized by a rash in the form of pinpoint macular lesions which may later coalesce. Rubella infection during pregnancy may lead to multisystemic disease and severe damage in the fetus. Consequently the diagnosis of Rubella during gestation is of considerable importance.

3 TEST PRINCIPLE SERION ELISA *classic*

The ELISA (Enzyme Linked Immunosorbent Assay) is an immunoassay which is particularly suited to the determination of antibodies. The reaction is based on the specific interaction of antibodies with their corresponding antigen. The test strips of the SERION ELISA *classic* microtiter plate are coated with specific antigens of the pathogen of interest. If antibodies in a sample are present, they bind to the fixed antigen. A secondary antibody, which has been conjugated with the enzyme alkaline phosphatase, detects and binds to the immune complex. The colorless substrate p-nitrophenylphosphate is then converted into the colored product p-nitrophenol. The signal intensity of this reaction product is proportional to the concentration of the analyte in the sample and is measured photometrically.

4 KIT COMPONENTS

Test components	Pieces / Volume
Break apart microtiter test strips each with 8 antigen coated single wells, (altogether 96) MTP, 1 frame. The coating material is inactivated	12 pieces
Standard serum (ready-to-use) STD Human serum in protein-containing phosphate buffer; negative for anti-HIV-Ab, HBs-Ag (Hepatitis B-Virus-surface antigen) and anti-HCV-Ab; preservative: < 0.1 % sodium azide; coloring: Amaranth O	2 x 2 ml
Negative control serum (ready-to-use) NEG Human serum in protein-containing phosphate buffer; negative for anti-HIV-Ab, HBs-Ag (Hepatitis B-Virus-surface antigen) and anti-HCV Ab; preservative: < 0.1 % sodium azide; coloring: Lissamin green V	2 ml
Anti-human-IgA, IgG, or IgM conjugate (ready-to-use) APC Anti-human-IgA, IgG, or IgM polyclonal antibody, conjugated to alkaline phosphatase, stabilized with protein stabilization solution; preservative: <0.1% methylisothiazolone, <0.1% bromnitrodioxane	13 ml
Washing solution concentrate (sufficient for 1000ml) WASH Sodium chloride solution with Tween 20 and 30 mM Tris/HCl, pH 7.4; preservative: < 0.1 % sodium azide	33.3 ml
Dilution buffer (ready-to-use) DILB Protein-containing phosphate buffer with Tween 20; preservative: < 0.1 % sodium azide; Coloring: 0.01 g/l Bromphenol blue	2 x 50 ml
Stopping solution (ready-to-use) STOP <0.1N sodium hydroxide, 40mM EDTA	15 ml
Substrate (ready-to-use) pNPP Para-nitrophenylphosphate in solvent free buffer; preservative: < 0.1 % sodium azide	13 ml
Quality control certificate with standard curve and evaluation table INFO (quantification of antibodies in IU/ml or U/ml)	1

5 MATERIAL REQUIRED BUT NOT SUPPLIED

- Common laboratory equipment
- For the IgM detection: SERION Rf-Absorbent (Order Nr. Z200, 20ml)
- Photometer for microtiter plates with filter, wavelength 405 nm, recommended reference wavelength 620 nm - 690 nm (e.g. 650 nm)
- Incubator 37°C
- Moist chamber
- Distilled water
- Click-Clips (Order Nr. VT120)
- Optional: SERION ELISA *control*

6 STORAGE AND STABILITY

Reagent	Storage	Stability
Microtiter strips (coated with antigen)	unopened after opening at 2-8°C in closed aluminum bag with desiccant	see expiry date on microtiter plate minimum shelf-life: 4 weeks
Control sera / Standard sera	unopened / after opening at 2-8°C	see expiry date
Conjugate	unopened / after opening at 2-8°C	see expiry date
Dilution buffer	unopened / after opening at 2-8°C	see expiry date
Washing solution	unopened / after opening at 2-8°C working dilution at 2-8°C working dilution at room temperature	see expiry date 2 weeks 1 week
Substrate	unopened / after opening at 2-8°C	see expiry date
Stopping solution	unopened /after opening at 2-8°C	see expiry date

7 TEST PROCEDURE SERION ELISA *classic*

7.1 Evidence of deterioration

Optimum results can only be achieved if the instructions are strictly followed. Only use SERION ELISA *classic* reagents when using SERION ELISA *classic* immunoassays. The components must not be exchanged for reagents of other manufacturers. Standard and control sera of SERION ELISA *classic* immunoassays are defined exclusively for the test kit to be used and must not be used in other lots. Washing solution, substrate, and stop solution can be used for all SERION ELISA *classic* immunoassays irrespective of the lot and the test.

Each SERION ELISA *classic* test contains a ready-to-use sample dilution buffer. In some cases the use of special dilution buffers is necessary to guarantee consistent quality and reliable results. The dilution buffers can be used irrespective of the lots.

There are three different conjugate concentrations for each immunoglobulin class (IgA, IgG, IgM) indicated on the label as + (low), ++ (medium) and +++ (high). Conjugates with the same concentration and of the same immunoglobulin class are interchangeable and can be used for other SERION ELISA *classic* immunoassays irrespective of the lot and the test. Dilution or alteration of the reagents may result in a loss of sensitivity. Use aseptic techniques when removing aliquots from the reagent tubes to avoid contamination.

Reproducibility of test results is dependent on thorough mixing of the reagents. Agitate the flasks containing control sera before use and also all samples after dilution (e.g. by using a vortex mixer).

Be sure to pipette carefully and comply with the given incubation times and temperatures. Significant time differences between pipetting the first and last well of the microtiter plate when dispensing samples and control sera, conjugate, or substrate can result in different pre-ioncubation times which may influence the precision and reproducibility of results. Avoid exposure of reagents to strong light during storage and incubation.

Adequate washing avoids test unspecificities. Therefore, the washing procedure should be carried out carefully. All of the flat bottom wells should be filled with equal volumes of washing buffer. At the end of the procedure ensure that the wells are free of all washing buffer in order to avoid uncontrolled dilution effects. Avoid foaming!

Reagents must be tightly closed after use to avoid evaporation and contamination. Take care not to mix up the caps of the bottles and/or vials.

The SERION ELISA *classic* immunoassay is only valid if the lot-specific validation criteria on the quality control certificate are fulfilled.

7.2 Sample preparation and storage

Lipaemic, hemolytic, or icteric samples (serum or plasma) should only be tested with caution. Obviously contaminated samples should not be tested. Serum or plasma (EDTA, citrate, heparin) or CSF collected according to standard laboratory methods are suitable samples. Samples must not be thermally inactivated.

7.2.1 Dilution of Samples

Before running the test, samples (V_1) must be diluted in dilution buffer (V_2) as follows:

SERION ELISA *classic* Rubella Virus IgG

$V_1 + V_2 = 1 + 100$	add	10 µl	sample
	each to	1000 µl	dilution buffer

SERION ELISA *classic* Rubella Virus IgM

Interference with rheumatoid factors

Rheumatoid factors are autoantibodies mainly of the IgM class which preferably bind to IgG immune complexes. The presence of non-specific IgM antibodies (rheumatoid factors) can lead to false-positive results in the IgM assay. Furthermore, the possibility exists that weak-binding pathogen-specific IgM antibodies may be displaced by stronger-binding IgG antibodies leading to a false-negative IgM result. Therefore, it is necessary to pretreat samples with rheumatoid factor absorbens prior to IgM detection (SERION Rf-Absorbent, Order Nr.:. Z200 (20 ml/100 tests)). Rf-absorption is performed by incubation of the sample in Rf-dilution buffer for 15 minutes at room temperature or overnight at 4°C. The test procedure is described in a separate instruction manual.

Before running the test, rheumatoid factor-absorbent (V_1) must be diluted 1+4 in dilution buffer (V_2).

$V_1 + V_2 = V3\ (1 + 4)$	add	200 µl	Rf-absorbent
	each to	800 µl	dilution buffer

Samples (V_4) must be diluted in this Rf-dilution buffer (V_3):

$V_4 + V_3 = 1 + 100$	add	10 µl	sample
	each to	1000 µl	Rf-dilution buffer

After dilution and before pipetting into the microtiter plate the samples must be mixed thoroughly to prepare a homogenous solution.

7.2.2 Sample storage

The samples should not be stored for more than 7 days at 2-8°C. Extended storage is possible at ≤ -20°C. Avoid repeated freezing and thawing of samples. Diluted samples can be stored at 2-8°C for one week.

7.3 Preparation of Kit Reagents

7.3.1 Microtiter Test Strips

The microtiter test strips labeled with abbreviations for pathogen and immunoglobulin class are packed with desiccant in an aluminum bag. To open the aluminum bag of the microtiter plate please cut off the top of the marked side only in order to guarantee proper resealing. Take unrequired cavities out of the frame and put them back into the aluminum bag. Close bag carefully to ensure airtight conditions. Do not use the strips if the aluminum bag is damaged or if the bag with remaining strips and desiccant was not properly resealed.

7.3.2 Control Sera / Standard Sera (ready-to-use)

Control and standard sera are ready-to-use and must not be diluted any further. For each test run – independent of the number of microtiter test strips to be used - control and standard sera must be included. Standard and cut-off sera should be set up in duplicate. Do not treat control sera with Rf-absorbent.

7.3.3 Anti-human IgA, IgG, or IgM AP-Conjugate (ready-to-use)

The required conjugate concentration (+, ++, +++) is indicated on the quality control certificate. Please refer also to the specification on the label.

7.3.4 Washing solution

Dilute washing buffer concentrate (V_1) 1:30 with distilled water to a final volume of V_2.

Example:

Buffer concentrate (V_1)	Final volume (V_2)
33.3 ml	1000 ml
1.0 ml	30 ml

7.3.5 Dilution Buffer for Samples (ready-to-use)

7.3.6 Substrate (ready-to-use)

Substrate in unopened bottle may have a slightly yellow coloring which does not reduce the quality of the product!

7.3.7 Stopping Solution (ready-to-use)

7.4 Overview - Test Procedure

SERION ELISA *classic*
Rubella Virus IgG/IgM
quantitative

In case of IgM detection absorption of rheumatoid factor, see No. 7.2.1;
Incubation 15 minutes at room temperature or over night at 4°C

sample dilution[1]
1+100

Pipette diluted samples and ready-to-use control sera /
standard sera into the microtest wells (100 µl)

⇩

INCUBATION 60 min./37°C
moist chamber

⇩

WASH (4 x 300µl DIL WASH) [2]

⇩

Pipette conjugate solution APC (100 µl)

⇩

INCUBATION 30 min./37°C
moist chamber

⇩

WASH (4 x 300µl DIL WASH) [2]

⇩

Pipette substrate solution pNPP (100 µl)

⇩

INCUBATION 30 min./37°C
moist chamber

⇩

Pipette stopping solution STOP (100 µl)

⇩

READ EXTINCTION AT 405 nm

[1]Special dilution buffers for the following SERION ELISA *classic* tests :
Borrelia burgdorferi IgG, IgM, EBV EA IgG, and Hantavirus Puumala IgG, IgM.

[2]For manual use:
tap plate at the end of the wash procedure on paper towel.

7.5 Manual Test Procedure

1. Place the required number of **cavities in the frame** and prepare a protocol sheet.

2. Add each **100µl of diluted sample or ready-to-use controls** into the appropriate wells of microtiter test strips. Spare one well for substrate blank, e.g.:

Well	Quantitative ELISA
A1	substrate blank
B1	negative control
C1	standard serum
D1	standard serum
E1	sample 1....
F1	sample 2…

3. **Sample incubation** for 60 minutes (+/- 5 min) at 37°C (+/- 1°C) in moist chamber**.**

4. After incubation **wash** all wells with washing solution (by automated washer or manually):
   - aspirate or shake out the incubation solution
   - fill each well with 300µl washing solution
   - aspirate or shake out the washing buffer
   - repeat the washing procedure 3 times (altogether 4 times!)
   - dry by tapping the microtest plate on a paper towel

5. **Addition of conjugate**
   Add 100µl of the ready-to-use IgG/IgM conjugate to the appropriate wells (except substrate blank).

6. **Conjugate incubation** for 30 minutes (+/- 1 min) * at 37°C (+/- 1°C) in moist chamber.

7. After incubation **wash** all wells with washing solution (see above).

8. **Addition of substrate**
   Add 100µl of ready-to-use substrate solution to each well (including well for substrate blank!).

9. **Substrate incubation** for 30 minutes (+/- 1 min) * at 37°C (+/- 1°C) in moist chamber.

10. **Stopping of the reaction**
    Add 100 µl stopping solution to each well, shake microtest plate gently to mix.

11. **Read extinction**

    Read optical density (OD) within 60 minutes at 405nm against substrate blank, reference wavelength between 620nm and 690nm (e.g. 650nm).

7.6 Automated Test Procedure

SERION ELISA are suited for processing on automats and evaluated for use with Immunomat™ and Gemini as well as with DYNEX DSX® and DS2®. The automated processing is performed analogous to manual use. Please note that under special working conditions, internal laboratory adaptations of the substrate incubation times may be necessary.

7.7 Positive Control / Accuracy Control

For the periodic verification of the test method, in order to fulfill the requirements of laboratory internal quality management systems, we recommend using SERION ELISA *controls* to determine precision and accuracy of SERION ELISA *classic* test runs. The use of SERION ELISA *controls* is described in specific instruction manuals.

7.8 Cerebral Spinal Fluid (CSF)

The SERION ELISA *classic* Rubella Virus IgG test has been evaluated for the determination of antibodies in CSF. The test procedure is described in a separate instruction manual. An Excel-based evaluation software tool supports the calculation of antibody indices according to a scheme of Prof. Hansotto Reiber.

7.9 Avidity Determination

The SERION ELISA *classic* Rubella Virus IgG test enables, in combination with the corresponding SERION ELISA *classic* Avidity Reagent, the avidity of pathogen-specific IgG antibodies to be determined. The test procedure is described in a separate instruction manual. The Excel-based evaluation software tool SERION *avidity* supports the calculation of SERION avidity indices.

8 TEST EVALUATION

8.1 SERION ELISA *classic* Rubella Virus IgG/IgM

The mathematical curve fitting for antibody quantification with SERION ELISA classic immunoassays is based on the 4-parameter logistic (4 PL) function.

$$Activity\ (U/ml) = e^{C-\frac{1}{B}\ln\left(\frac{D-A}{OD(Patient)*F-A}-1\right)}$$

The 4 parameters A, B, C, and D are representative for the exact shape of the standard curve:

Parameter A:	Lower asymptote (OD)
Parameter B:	Slope of the curve
Parameter C:	Inflection point
Parameter D:	Upper asymptote (OD)

Institut Virion\Serion GmbH established a lot-specific 4 PL standard curve for each SERION ELISA *classic* immunoassay in multiple test runs under optimal test conditions. The four parameters are indicated on the quality control certificate of each individual SERION ELISA *classic* test.

For the adaptation of the test level to the given 4 PL standard curve the correction factor F is calculated by dividing the standard reference OD value indicated on the quality control certificate with the measured, and consequently test run-specific, standard OD value:

$$F = \frac{\textit{STD reference OD value}}{\textit{measured STD OD value}}$$

By multiplying the OD values obtained from samples with the correction factor F, the level of each individual test run is adjusted to the given 4 PL standard curve. Thereby, interassay deviations are compensated for, and antibody activities can be directly evaluated from the 4 PL standard curve.

After subtraction of the substrate blank from all measured OD values and calculation of the mean OD value of the standard serum (STD), tested in duplicate, a range of possibilities are available for the evaluation of antibody activities from the optical measurement signals (OD) of samples. They are described in separate manuals.

The determination of the IgG antibody activity in IU/ml is referenced to the International Standard Anti-Rubella Immmunoglobulin (NIBSC code RUBI-1-94) of the World Health Organization (WHO). The determination of the IgM antibody activity in U/ml is referenced to the Rubella Virus IgM Standard Serum Leipzig, Germany.

8.2 Borderline Ranges

The borderline ranges of the SERION ELIA *classic* Rubella Virus IgG/IgM tests are specified on the quality control certificates and indicate the range of borderline test results. Values below this range indicate negative values; values above the borderline range indicate positive values.

8.3 Limits of Quantification

The limits of quantification are specified on the quality control certificate of the SERION ELISA *classic* Rubella Virus IgG/IgM. The linearity of dilution within this range has been demonstrated in comprehensive evaluation studies. If a sample shows a test result above the upper limit of quantification, the sample may be tested at a higher dilution. The resulting antibody activity must then be multiplied by the additional dilution factor.

8.4 Automated Evaluation / Software

For the automated evaluation of optical measurement signals, the Software SERION easy*ANALYZE*, the software SERION *evaluate*, as as the Microsoft® Excel®-based software tool SERION *activity* are available on request.

8.5 Criteria of Validity

- The substrate blank must be < 0.25 OD.
- The negative control must be negative
- By use of quantitative SERION ELISA *classic* tests the mean OD-value (after subtraction of the substrate blank!) of the standard serum must be within the validity range which is given on the lot specific quality control certificate of the kit.

- By use of qualitative SERION ELISA *classic* tests the OD value of the positive control and the mean OD value of the cut-off serum must be within the validity range which is given on the lot specific quality control certificate of the kit (after subtraction of the substrate blank!).
- The variation of OD values of the standard serum or cut-off serum must not be higher than 20%.

If these criteria are not met, the test is not valid and must be repeated.

9. SAFETY MEASURES

9.1 Statements of Warning

- The SERION ELISA classic is designed for use by qualified personnel who are familiar with good laboratory practice. All kit reagents and samples should be handled carefully using established good laboratory practice.
- This kit contains human blood components. Although all control and cut-off sera have been tested and found negative for anti-HIV Ab, HBsAg (Hepatitis B-Virus-surface Antigen), and anti-HCV Ab, they should be considered potentially infectious.
- Do not pipette by mouth.
- Do not smoke, eat, or drink in areas in which samples or kit reagents are handled.
- Wear disposable gloves, laboratory coat, and safety glasses while handling kit reagents or samples. Wash hands thoroughly afterwards.
- Samples and other potentially infectious material should be decontaminated after the test run.
- Reagents should be stored safely and be inaccessible to unauthorized access, e.g. children.

9.2 Disposal

Please observe the relevant statutory requirements!