SERION ELISA *classic* Adenovirus IgA/IgG/IgM

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SERION ELISA *classic* Adenovirus IgG/IgA

Enzyme Immunoassay for detection of human antibodies
- For sale in the U.S. for Research Use Only. Not for diagnostic procedures.

SERION ELISA *classic* Adenovirus IgA	order number: ESR128A
SERION ELISA *classic* Adenovirus IgG	order number: ESR128G
SERION ELISA *classic* Adenovirus IgM	order number: ESR128M

1. INTENDED USE

SERION ELISA *classic* Adenovirus IgA/IgG/IgM tests are immunoassays for the detection of human antibodies in serum or plasma directed against all pathogenic human adenovirus serotypes.

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

2. BACKGROUND

In the 1950s Adenoviruses were described as latent persisting agents in tonsils and adenoid tissue, as well as the cause of acute respiratory tract infection. Adenoviruses cause diseases in multiple organ systems (respiratory tract, gastrointestinal tract, eye- and urogenital tract).

Adenoviruses belong to the family of adenoviridae and are icosahedral particles with a size of 90-100nm. The virus consists of a protein capsid containing double-stranded DNA. Adenoviruses that are pathogenic for humans consist of 51 serotypes sorted into several subtypes. Serotype 1-8, 40, and 41 are most commonly isolated from humans.

3. SERION ELISA *classic* - TEST PRINCIPLE

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 antigen-antibody 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 antibody in the sample and is measured photometrically.

4. COMPONENTS OF THE KIT

Test components	Pieces / Volume	
	IgA/IgG	**IgM**
Break apart microtiter test strips each with 8 antigen coated single wells (altogether 96) MTP 1 frame. The coating material is inactivated.	12 pieces	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 2ml	-
Cut-off serum (ready-to-use) C/O 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: Chinaldin yellow	-	2 x 2ml
Positive control serum (ready-to-use) POS 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	-	2ml
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: Lissamine green V	2ml	2ml
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	13ml	13ml
Washing solution concentrate (sufficient for 1000ml) WASH Sodium chloride solution with Tween 20, 30mM Tris-HCl, pH 7.4; preservative: < 0.1% sodium azide	33.3ml	33.3ml
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 50ml	2 x 50ml
Stopping solution (ready-to-use) STOP <0.1N sodium hydroxide, 40mM EDTA	15ml	15ml
Substrate (ready-to-use) pNPP Para-nitrophenylphosphate in solvent free buffer; preservative: < 0.1 % sodium azide	13ml	13ml
Quality control certificate with standard curve and evaluation table INFO (quantification of antibodies in IU/ml or U/ml)	2 pages	-
Quality control certificate INFO	-	1 page

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Common laboratory equipment

- For the IgM-ELISA: SERION Rf-Absorbent (Order no. Z200/20ml)

- Photometer for microtiter plates with filter, wave length 405nm, recommended reference wave length 620nm - 690nm (e.g. 650nm)
- Microtiter plate washer
- Incubator 37°C
- Moist chamber

- Distilled water

- 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 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 for 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 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-incubation times which may influence the precision and reproducibility of the 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) 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* Adenovirus IgA

$V_1 + V_2 = 1 + 500$	add each to	10 µl sample 1000µl dilution buffer (= 1:100)
	each to	50µl from the first dilution step 200µl dilution buffer (= 1:4)

SERION ELISA *classic* Adenovirus IgG

$V_1 + V_2 = 1 + 2000$	add each to	10µl sample 1000µl dilution buffer (= 1:100)
	each to	50µl from first dilution step 950µl dilution buffer (= 1:19)

SERION ELISA *classic* Adenovirus IgM

Interference by rheumatoid factors
Rheumatoid factors are autoantibodies mainly of the IGM class which preferentially 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-absorbent prior to IgM detection (SERION Rf-Absorbent, order no. Z200 (20ml/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).

$V_1 + V_2 = V_3$ (1:4)	add	200ul Rf-absorbent
	each to	800ul dilution buffer

Samples (V4) must be diluted in this Rf-dilution buffer (V_3):

V4 + V_3 = 1:100	add	10ul sample
	each to	1000ul 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

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

Bring all reagents to room temperature before use.

7.3.1 Microtiter Test Strips
The microtiter test strips labeled with abbreviations for pathogen and immunoglobulin class are packed with a 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 unneeded wells/strips out of the frame and put them back in 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 microtest 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, IgM or 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 (Concentrate)
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 slight yellow color 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*
Adenovirus IgA/IgG quantitative
Adenovirus IgM quantitative**

sample dilution[1]
IgA : 1:500
IgG: 1:2000
IgM: 1:100
⇓

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

| INCUBATION 60 min./ 37°C |
| moist chamber |
⇓

WASH (4 x 300ul DIL WASH)[2]
⇓

Pipette conjugate solution APC (100µl)
⇓

| INCUBATION 30 min./ 37°C |
| moist chamber |
⇓

WASH)4 x 300ul DIL WASH)[2]
⇓

Pipette substrate solution pNPP (100µl)
⇓

| INCUBATION 30 min./ 37°C |
| moist chamber |
⇓

Pipette stopping solution STOP (100µl)
⇓

| 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 **wells 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 microtest strips. Spare one well for substrate blank, e.g.:

Well	Quantitative ELISA	Qualitative ELISA
A1	substrate blank	substrate blank
B1	negative control	negative control
C1	standard serum	cut off – serum
D1	standard serum	cut off – serum
E1	sample 1 . . .	positive control
F1	sample 2 . . .	sample 1 . . .

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 IgA/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 100µl stopping solution to each well, shake microtiter plate gently to mix.

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

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.

8. TEST EVALUATION

8.1 SERION ELISA *classic* Adenovirus IgA/IgG

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

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

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

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

Institut Virion\Serion GMbH establishes 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.

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

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 4PL standard curve.

8.2   Borderline Ranges

The borderline ranges of the SERION ELISA classic Adenovirus IgA/IgG tests are specified on the quality control certificates and indicate the range of borderline assay results. Values below this range indicate a negative assay result; values above the borderline range indicate a positive assay result.

8.3   Limits of Quantification

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

8.4   SERION ELISA classic Adenovirus IgM

For the evaluation of test runs a lot-specific quality control certificate with declarations concerning cut-off serum and positive control is included in every SERION ELISA *classic*.

Before evaluation, the blank value (blank) has to be subtracted from each sample value. For determination of the cut-off range in OD the mean of the readings for the cut-off serum has to be calculated. The cut-off range in OD corresponds to the mean value of the cut off serum +/- 10%.

OD sample	more than	10% over	OD cut-off	positive value
OD sample	+/-	10% of	OD cut-off	borderline value
OD sample	more than	10% under	OD cut-off	negative value

8.5   Automated Evaluation / Software

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

8.6 Criteria of Validity

- The substrate blank must be OD < 0.25

- 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.

- By use of qualitative SERION ELISA *classic* tests the mean OD-value of the positive control and the mean OD value of the cut-off serum must be within the validity ranges, which are 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. STATEMENTS OF WARNING

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 human 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 antibody, HBs-Ag (*Hepatitis B-Virus surface Antigen*), and HCV antibody, 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.

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- 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!

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