# VIROTECH Adenovirus IgG/IgM ELISA
## (Adenovirus IgG/IgM ELISA)

**Order No.: EC121.00**

# Adenovirus IgA-Set

**Order No.: EC121.08**

**Color Coding: dark blue/transparent**

**FOR IN VITRO DIAGNOSIS ONLY**

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# Contents

## 1. Intended Use

The ELISA is intended for the qualitative and semiquantitative detection of IgG-, IgM- and IgA-antibodies against Adenovirus in human serum.

## 2. Diagnostic Relevance

Human Adenoviruses belong to the genus mastadenovirus of the family of adenoviridae (1).
They are non-enveloped viruses with linear doublestrand DNA and are grouped into the subgenera A-F. 49 serotypes are described until now (2) but only a few cause real infectious diseases. Most of them remain subclinical (3).
Transmission happens either by faecal-oral route, by aerosol or by ophthalmologistôs instruments (epidemic keratoconjunctivitis). Incubation time is 5-8 days.
Adenoviruses cause diseases of the upper and lower respiratory tract, gastroenteritis in children (type 40 and 41, second frequent gastroenteritis pathogen after rotaviruses), epidemic keratoconjunctivitis (types 8, 19 and 37) and acute haemorrhagic cystitis (type 11).
A long lasting serotypespecific immunity is kept after an infection (2).
There is no specific immunologic prevention or therapy available until now, although it is especially searched for antiviralia intensively (4).
Differential diagnostically Parainfluenza viruses, Adenoviruses, RSV, Influenza viruses, rhinoviruses, enteroviruses or Chlamydia trachomatis resp. Pneumocystis carinii, if symptoms of immunosupprussion are present, are possible for acute infections of the lower respiratory tract (5).
The Antigen detection is performed when gastroenteritis, keratoconjunctivitis and respiratory infects are suspected. The non-typespecific ELISA is used for supportive diagnosis of respiratory infects (6).

## 3. Test Principle

The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.

## 4. Package Contents

### 4.1 IgG/IgM Testkit

1. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised
2. **PBS-Dilution Buffer (blue, ready to use) 2x50ml**, pH 7,2, with preservative and Tween 20
3. **PBS-Washing Solution (20x concentrated) 50ml**, pH 7,2, with preservative and Tween 20
4. **IgG negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
5. **IgG cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
6. **IgG positive Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
7. **IgM negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
8. **IgM cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
9. **IgM positive Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
10. **IgG-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with protein-stabilizer and preservative in Tris-Buffer, ready to use
11. **IgM-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use
12. **Tetramethylbenzidine substrate solution (3,3ô5,5ôTMB), 11ml**, ready to use
13. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

### 4.2 IgA Set

1. **IgA negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
2. **IgA cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
3. **IgA positive Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
4. **IgA-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use

## 5. Storage and Shelflife of the Testkit and the ready to use reagents

Store the testkit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. Microtiter strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not useable anymore.
3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

| Material | Status | Storage | Shelflife |
|---|---|---|---|
| Test Samples | Diluted | +2 to +8°C | max. 6h |
| | Undiluted | +2 to +8°C | 1 week |
| Controls | After Opening | +2 to +8°C | 3 months |
| Microtitreplate | After Opening | +2 to +8° (storage in the provided bag with desiccant bag) | 3 months |
| Rheumatoid factor - Absorbent | Undiluted, After Opening | +2 to +8°C | 3 months |
| | Diluted | +2 to +8°C | 1 week |
| Conjugate | After Opening | +2 to +8°C (protect from light) | 3 months |
| Tetramethylbenzidine | After Opening | +2 to +8°C (protect from light) | 3 months |
| Stop Solution | After Opening | +2 to +8°C | 3 months |
| Washing Solution | After Opening | +2 to +8°C | 3 months |
| | Final Dilution (ready-to-use) | +2 to +25°C | 4 weeks |

## 6. Precautions and Warnings

1. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls, conjugates and microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If body parts are contacted, immediately wash them under flowing water and possibly consult a doctor.
3. The disposal of the used materials has to be done according to the country-specific guidelines.

## 7. Material required but not supplied

1. Aqua dest./demin.
2. Eight-channel pipette 50µl, 100µl
3. Micropipettes: 10µl, 100µl, 1000µl
4. Test tubes
5. Paper towels or absorbent paper
6. Cover for ELISA-plates
7. Disposal box for infectious material
8. ELISA handwasher or automated EIA plate washing device
9. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
10. Incubator

## 8. Test Procedure

Working exactly referring to the VIROTECH Diagnostics user manual is the prerequisite for obtaining correct results.

### 8.1 Examination Material

Either serum or plasma can be used as test material, even if only serum is mentioned in the instructions. Any type of anticoagulant can be used for plasma.

Always prepare patient-dilution freshly.

For a longer storage the sera must be frozen. Repeated defrosting should be avoided.

1. Only fresh non-inactivated sera should be used.
2. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera should not to be used (false positive/negative results).

### 8.2 Preparation of Reagents

The VIROTECH Diagnostics System Diagnostica offers a high degree of flexibility regarding the possibility to use the dilution buffer, washing solution, TMB, citrate stopping solution as well as the conjugate for all parameters and for all different lots. The ready to use controls (positive control, negative control, cut-off control) are **parameter specific** and **only to use** with the plate lot indicated in the Quality Control Certificate.

5. Set incubator to 37°C and check proper temperature setting before start of incubation.
6. Bring all reagents to room temperature before opening package of microtiter strips.
7. Shake all liquid components well before use.
8. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.
9. High IgG-titer or rheumatoid factors may disturb the specific detection of IgM-antibodies and may lead to false positive resp. false negative results. **For a correct IgM-determination it is therefore necessary to pre-treat the sera with RF-SorboTech** (VIROTECH adsorbent). For IgM-controls a pre-absorbent treatment is not necessary.

### 8.3 VIROTECH ELISA Test Procedure

1. For each test run, pipette 100µl each of ready to use dilution buffer (blank), IgG- IgM- and IgA-positive, negative and cut-off controls as well as diluted patient sera. We propose a double insertion (blank, controls and patient sera); for cut-off control a double insertion is absolutely necessary. Working dilution of patient sera: 1+100; e.g. 10µl serum + 1ml dilution buffer.
2. After pipetting start incubation for 30 min. at 37°C (with cover).
3. End incubation period by washing microtiter strips 4 times with 350 ï 400µl washing solution per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100µl of ready to use conjugate into each well.
5. Incubation of conjugates: 30 min. at 37°C (with cover).
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark).
9. Stopping of substrate reaction: pipette 50µl of citrate stopping solution into each well. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to last page for Test Procedure Scheme

### 8.4 Usage of ELISA processors

All VIROTECH Diagnostics ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.

VIROTECH Diagnostics recommends the following procedure:

1. VIROTECH Diagnostics recommends to proceed the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.
2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterwards. A regular check using the Validationkit shall be proceeded minimum once a quarter to test the accuracy of the processor.
3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each testrun.

With this procedure, your ELISA processor will function properly and this will support quality assurance in your laboratory.

## 9. Test Evaluation

The ready to use controls serve for a semiquantitative determination of specific IgG-, IgA- and IgM-antibodies. Their concentration can be expressed in VIROTECH units = VE. Fluctuations resulting from the test procedure can be balanced with this calculation method and a high reproducibility is achieved in this way. Use the means of the OD values for calculation of the VE.

### 9.1 Test function control

a) OD-values

The OD of the blank should be < 0.15.

The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.

b) VIROTECH Units (VE)

The VIROTECH Units (VE) of the cut-off controls are defined as 10 VE. The calculated VE of the positive controls should be within the ranges mentioned in the Quality Control Certificate.

If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

### 9.2 Calculation of the VIROTECH Units (VE)

The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

$$VE_{(positive\ control)} = \frac{OD_{(positive\ control)}}{OD_{(cut\text{-}off\ control)}} \times 10$$

$$VE_{(patient\ serum)} = \frac{OD_{(patient\ serum)}}{OD_{(cut\text{-}off\ control)}} \times 10$$

### 9.3 Interpretation Scheme IgG, IgM and IgA

| Result (VE) | Evaluation |
|---|---|
| < 9,0 | negative |
| 9,0 - 11,0 | borderline |
| > 11,0 | positive |

1. If the measured values are above the defined borderline range, they are considered to be positive.
2. If the measured VE is within the borderline range, no significant high antibody concentration is present, the samples are considered to be borderline. For the secure detection of an infection it is necessary to determine the antibody concentration of two serum samples. One sample shall be taken directly at the beginning of the infection and a second sample 5 ï 10 days later (convalescent serum). The antibody concentration of both samples has to be tested in parallel, that means in one test run. A correct diagnosis based on the evaluation of a single serum sample is not possible.
3. If the measured values are below the defined borderline range, no measurable antigen specific antibodies are present in the samples. The samples are considered to be negative.
4. A positive IgG result indicates either a past infection or a recent infection.
   A positive IgM result indicates an acute infection and a positive IgA result indicates a relatively acute infection, as IgA can persist for months.

A negative result indicates, that the patient is/was not infected.

### 9.4 Limits of the Test

1. The interpretation of serological results shall always include the clinical picture, epidemiological data and all further available laboratory results.
2. Anti-doublestrand DNA ($\alpha$-dsDNA) sera (ANA, systemic lupus erithematodes) show cross reactivities to the VIROTECH Diagnostics Adenovirus ELISA.
3. Please pay attention to the following:
   In exceptional cases it might come to false positive results due to crossreactions with other pathogens or due to unusually high concentrations of sera parts.
   False negative results may occur in non-responders, in immunosuppressed patients or if the sample is taken too early.

## 10. Performance Data

### 10.1 Sensitivity and Specificity

To determine the sensitivity and specificity sera were tested in the Virtotech ELISA and in an ELISA of a competitor.

Seracollective (IgG n=300, IgM n=286)

| Competitor | **VIROTECH Adenovirus ELISA** | | | |
|---|---|---|---|---|
| | IgG | | IgM | |
| | negative | positive | negative | positive |
| negative | 91 | 18 | 190 | 3 |
| positive | 0 | 164 | 5 | 62 |

Borderline results have not been included into the calculation of sensitivity and specificity. The values for the VIROTECH Adenovirus ELISA are:

| | IgG | IgM |
|---|---|---|
| Sensitivity | >99,8% | 92,5% |
| Specificity | 83,5% | 98,4% |

### 10.2 Prevalence (Expected Values)

The following table shows the results of the examination of blood bank sera for IgG (n=119), IgM (n=80) and IgA (n=119):

| | IgG | IgM | IgA |
|---|---|---|---|
| negative | 6 | 80 | 90 |
| borderline | 7 | 0 | 10 |
| positive | 106 | 0 | 19 |

### 10.3 Intra-assay-Coefficient of Variation (Repeatability)

In one assay, strips of different plates of one batch have been tested with the same serum sample. The obtained coefficient of variation is < 9% (at an average OD-value of 0,37).

### 10.4 Inter-assay-Coefficient of Variation (Reproducibility)

Three sera were tested in 12 independent test runs by different persons in different laboratories.

**Adenovirus ELISA IgG**

| Serum | Average Value VE | Coefficient of Variation |
|---|---|---|
| **negative** | 8,1 | 6,2% |
| **positive** | 13,9 | 4,9% |
| **positive** | 31,2 | 6,2% |

## 11. Literature


1. Thomas Porstmann (Hrsg.), Virusdiagnostik, Diagnostische Bibliothek Band 1, Blackwell Wissenschaft 1996, S. 104
2. http://virologie.medizin.uni-essen.de/html/analysen/ana_adeno.htm erstellt von Dr. R. Scheidhauer, letzte Änderung: 21.05.2002
3. http://www.vu-wien.ac.at/i123/SPEZVIR/ADENOGEN1.HTML (Veterinärmedizinisches Institut der Universität Wien), Stand Juni 2003
4. Thomas Porstmann (Hrsg.), Virusdiagnostik, Diagnostische Bibliothek Band 1, Blackwell Wissenschaft 1996, S. 113
5. Brandis, Köhler, Eggers, Pulverer, Lehrbuch der Medizinischen Mikrobiologie, 7. Auflage, Fischer 1994, S.839
6. Mikrobiologische Diagnostik und Krankenhaushygiene, MVP, 2. Ausgabe, Stand Jan 2003, S. 48 und 64

# Preparation of Patient Samples and Washing Solution

- **Washing Solution:** Fill up concentrate to 1 liter with aqua dest./demin.

- **IgG-/IgA-Samples - Dilution 1:101**

e.g.:
10 µl serum/plasma + 1000 µl Dilution Buffer
(Serum Dilution Buffer is ready to use)

- **IgM-Samples - Dilution 1:101**
  **Rheumafactor-absorption with RF-SorboTech**

e.g.:
5 µl serum/plasma + 450 µl Dilution Buffer +
1 drop RF-SorboTech, incubate for 15 min. at room temperature.

# Testprocedure

| | | |
|---|---|---|
| Samples Incubation | **30 minutes at 37°C** | **100 µl Patient Samples**<br>blank value (Dilution Buffer) and controls |
| ⇩ | | |
| Wash 4times | | **400 µl Washing Solution**<br>Remove Residues on a Cellulose Pad |
| ⇩ | | |
| Conjugate Incubation | **30 minutes at 37°C** | **100 µl Conjugate**<br>IgG, IgM, IgA |
| ⇩ | | |
| Wash 4times | | **400 µl Washing Solution**<br>Remove Residues on a Cellulose Pad |
| ⇩ | | |
| Substrate Incubation | **30 minutes at 37°C** | **100 µl Substrate** |
| ⇩ | | |
| Stopping | | **50 µl Stopping Solution**<br>shake carefully |
| ⇩ | | |
| Measure Extinctions | | **Photometer at 450/620nm**<br>**(Reference Wavelength 620-690nm)** |