Version 11-01/25

User's Manual

Adenovirus IgM ELISA Kit

REF **DEIA1767-1**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This test is immunoassay for the detection of human antibodies in serum or plasma directed against all pathogenic human adenovirus serotypes.

General Description

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.

Principles of Testing

The test strips of the ELISA 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.

Reagents And Materials Provided

1. Break apart microtiter test strips each with 8 antigen coated single wells (altogether 96), 1 frame. The coating material is inactivated.

2. Cut-off serum (ready-to-use), 2 × 2ml. 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

3. Positive control serum (ready-to-use), 2ml. 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

4. Negative control serum (ready-to-use), 2ml. 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

5. Anti-human-IgA, IgG, or IgM conjugate (ready-to-use), 13ml. Anti-human-IgA, IgG, or IgM polyclonal antibody, conjugated to alkaline phosphatase, stabilized with protein stabilization solution; preservative: 0.1% methylisothiazolone, <0.1 % bromnitrodioxane

6. Washing solution concentrate (sufficient for 1000ml), 33.3ml. Sodium chloride solution with Tween 20,

30mM Tris-HCl, pH 7.4; preservative: < 0.1% sodium azide

7. Dilution buffer (ready-to-use), 2 × 50ml. Protein-containing phosphate buffer with Tween 20; preservative: < 0.1% sodium azide; coloring: 0.01 g/l Bromphenol blue.

8. Stopping solution (ready-to-use), 15ml. <0.1N sodium hydroxide, 40mM EDTA

9. Substrate (ready-to-use), 13ml. Para-nitrophenylphosphate in solvent free buffer; preservative: < 0.1 % sodium azide

Materials Required But Not Supplied

1. Common laboratory equipment
2. For the IgM-ELISA: Rf-Absorbent
3. Photometer for microtiter plates with filter, wave length 405nm, recommended reference wave length 620nm - 690nm (e.g. 650nm)
4. Microtiter plate washer
5. Incubator 37°C
6. Moist chamber
7. Distilled water
8. Optional: ELISA control

Storage

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

Specimen Collection And Preparation

1. 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.

a. Dilution of Samples

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 factorabsorbent prior to IgM detection (Rf-Absorbent). Rfabsorption 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 (V_4) must be diluted in this Rf-dilution buffer (V_3):

V4 + V3 = 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.

b. 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.

Reagent Preparation

Bring all reagents to room temperature before use.

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.

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.

3. Anti-human 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.

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

5. Dilution buffer for samples (ready-to-use)

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. Stopping solution (ready-to-use)

Assay 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	Qualitative ELISA
A1	substrate blank
B1	negative control
C1	cut off – serum
D1	cut off – serum
E1	positive control
F1	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).

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 ELISA controls to determine precision and accuracy of ELISA classic test runs. The use of ELISA controls is described in specific instruction manuals.

Calculation

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 ELISA.

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

Criteria of Validity

1. The substrate blank must be OD < 0.25
2. The negative control must be negative
3. By use of quantitative ELISA 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.
4. By use of qualitative ELISA 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!)
5. 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.

Precautions

1. Evidence of deterioration

Optimum results can only be achieved if the instructions are strictly followed. Only use CD ELISA reagents when using CD ELISA immunoassays. The components must not be exchanged for reagents of other manufacturers. Standard and control sera of ELISA immunoassays are defined exclusively for the test kit to be used and must not be used in other lots.

Each ELISA test contains a ready-to-use sample dilution buffer.

There are three different conjugate concentrations for each immunoglobulin class (IgA, IgG, IgM) indicated on the label as + (low), ++ (medium) and +++ (high). 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 ELISA immunoassay is only valid if the lot-specific validation criteria on the quality control certificate are fulfilled.

Statements of warning

The ELISA 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.

1. 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.
2. Do not pipette by mouth.
3. Do not smoke, eat or drink in areas in which samples or kit reagents are handled.
5. Wear disposable gloves, laboratory coat and safety glasses while handling kit reagents or samples. Wash hands thoroughly afterwards.
6. Samples and other potentially infectious material should be decontaminated after the test run.
7. Reagents should be stored safely and be inaccessible to unauthorized access e.g. children.