Adenovirus IgM ELISA Kit

Cat. No.:DEIA1767
Pkg.Size:96T

Intended use

The Adenovirus IgG Enzyme Immunoassay Kit provides materials for the qualitative and semiquantitative determination of IgG-class antibodies to Adenovirus in serum.

General Description

Adenoviruses are medium-sized (90–100 nm), nonenveloped (without an outer lipid bilayer) icosahedral viruses composed of a nucleocapsidand a double-stranded linear DNA genome. There are 57 described serotypes in humans, which are responsible for 5–10% of upper respiratory infections in children, and many infections in adults as well.
Viruses of the family Adenoviridae infect various species of vertebrates, including humans. Adenoviruses were first isolated in 1953 from human adenoids. They are classified as group I under the Baltimore classification scheme, meaning their genomes consist of double stranded DNA.

Principle Of The Test

The Adenovirus IgG ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) Microtiter wells as a solid phase are coated with Adenovirus antigen. Diluted patient specimens and ready-for-use controls are pipetted into these wells. During incubation Adenovirusspecific antibodies of positive specimens and controls are bound to the immobilized antigens. After a washing step to remove unbound sample and control material horseradish peroxidase conjugated anti-human IgG antibodies are dispensed into the wells. During a second incubation this anti-IgG conjugate binds specifically to IgG antibodies resulting in the formation of enzyme-linked immune complexes. After a second washing step to remove unbound conjugate the immune complexes formed (in case of positive results) are detected by incubation with TMB substrate and development of a blue color. The blue color turns into yellow by stopping the enzymatic indicator reaction with sulfuric acid. The intensity of this color is directly proportional to the amount of Adenovirus-specific IgG antibody in the patient specimen. Absorbance at 450 nm is read using an ELISA microtiter plate reader.

Reagents And Materials Provided

1. Microtiterwells, 12 x 8 (break apart) strips, 96 wells;
Wells coated with Adenovirus antigen.
(incl. 1 strip holder and 1 cover foil)
2. Sample Diluent *, 1 vial, 100 mL, ready to use,
colored yellow; pH 7.2 ± 0.2.
3. Pos. Control *, 1 vial, 1.0 mL, ready to use;
colored yellow, red cap.
4. Neg. Control *, 1 vial, 2.0 mL, ready to use;
colored yellow, yellow cap.
5. Cut-off Control *, 1 vial, 2.0 mL, ready to use;
colored yellow, black cap.
6. Enzyme Conjugate *, 1 vial, 20 mL, ready to use,
colored red,
antibody to human IgG conjugated to horseradish peroxidase.

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7. Substrate Solution, 1 vial, 14 mL, ready to use,
Tetramethylbenzidine (TMB).
8. Stop Solution, 1 vial, 14 mL, ready to use,
contains 0.2 mol/L H_2SO_4,
Avoid contact with the stop solution. It may cause skin irritations and burns.
9. Wash Solution *, 1 vial, 30 mL (20X concentrated for 600 mL), pH 6.5 ± 0.1

Materials Required But Not Supplied

1. A microtiter plate calibrated reader (450/620nm ±10 nm)
2. Calibrated variable precision micropipettes
3. Incubator 37 °C
4. Manual or automatic equipment for rinsing wells
5. Vortex tube mixer
6. Deionised or (freshly) distilled water
7. Timer
8. Absorbent paper

Storage

1. When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
2. Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
3. Opened kits retain activity for four months if stored as described above.

Specimen Collection And Handling

Serum can be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.

Serum:

Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Specimen Storage

Specimens should be capped and may be stored for up to 24 hours at 2 °C to 8 °C prior to assaying. Specimens held for a longer time should be frozen only once at –20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

Specimen Dilution

Prior to assaying dilute each patient specimen 1±1000 with Sample Diluent;
step 1: 10 µL of specimen ± 1 mL of Sample Diluent
step 2: 25 µL of prediluted specimen (step 1) ± 250 µL of Sample Diluent
mix well, let stand for 15 minutes mix well before use.

Reagent Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.

Wash Solution

Dilute Wash Solution 1±19 (e.g. 10 mL ± 190 mL) with fresh and germ free redistilled water. This diluted wash solution has a pH value of 7.2 ± 0.2. Consumption: ~ 5 mL per determination. Crystals in the solution disappear by warming up to 37 °C in a water

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bath. Be sure that the crystals are completely dissolved before use. The diluted Wash Solution is stable for 4 weeks at 2 °C to 8 °C.

Assay Steps

General Remarks

1. Please read the test Protocol carefully before performing the assay. Result reliability depends on strict adherence to the test Protocol as described.
2. It is very important to bring all reagents, samples and controls to room temperature before starting the test run!
3. Once the test has been started, all steps should be completed without interruption.
4. Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
5. Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
6. As a general rule the enzymatic reaction is linearly proportional to time and temperature.
7. Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
8. After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
9. To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
10. During incubation cover microtiter strips with foil to avoid evaporation.

Assay procedure

Prior to commencing the assay, dilute Wash Solution, prepare patient samples as described in point 5.3, mix well before pipette and establish carefully the distribution and identification plan supplied in the kit for all specimens and controls.

1. Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:

1 well (e.g. A1) for the substrate blank,
1 well (e.g. B1) for the Neg. Control,
2 wells (e.g. C1±D1) for the Cut-off Control and
1 well (e.g. E1) for the Pos. Control.

It is left to the user to determine controls and patient samples in duplicate.

2. Dispense

100 µL of Neg. Control into well B1
100 µL of Cut-off Control into wells C1 and D1
100 µL of Pos. Control into well E1 and
100 µL of each d i l u t e d sample with new disposable tips into appropriate wells.

Leave well A1 for substrate blank!

3. Cover wells with foil supplied in the kit. Incubate for 60 minutes at 37 °C.
4. Briskly shake out the contents of the wells. Rinse the wells 5 times with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets. Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
5. Dispense 100 µL Enzyme Conjugate into each well, except A1.
6. Cover wells with foil. Incubate for 30 minutes at room temperature (20 °C to 25 °C). Do not expose to direct sun light!
7. Briskly shake out the contents of the wells. Rinse the wells 5 times with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
8. Add 100 µL of Substrate Solution into all wells.
9. Cover wells with foil. Incubate for exactly 15 minutes at room temperature (20 °C to 25 °C) in the dark.
10. Stop the enzymatic reaction by adding 100 µL of Stop Solution to each well.

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Any blue color developed during the incubation turns into yellow.
Note: Highly positive patient samples can cause dark precipitates of the chromogen!
11. Read the optical density at 450/620 nm with a microtiter plate reader within 30 minutes after adding the Stop Solution.

Measurement

Adjust the ELISA microplate or microstrip reader to zero using the substrate blank in well A1.
If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!
Measure the absorbance of all wells at 450 nm and record the absorbance values for each control and patient sample in the distribution and identification plan.
Dual wavelength reading using 620 nm as reference wavelength is recommended.
Where applicable calculate the mean absorbance values of all duplicates.

Quality Control

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

Calculation

Mean absorbance value of Cut-off Control [CO]
Calculate the mean absorbance value of the two Cut-off Control determinations (e.g. in C1/D1).
Example: (0.59 ± 0.61) : 2 = 0.60 = CO

Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 100 %.

Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 100 %.

Precautions

1. Please use only the valid version of the package insert provided with the kit.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II,
3. HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
4. Controls and Standards has been found to be non-infectious in cell cultures.
5. Avoid contact with Stop Solution containing 0.2 mol/L H_2SO_4. It may cause skin irritation and burns.
6. Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
7. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
8. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
9. Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or

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regulation.
10. Do not use reagents beyond expiry date as shown on the kit labels.
11. All indicated volumes have to be performed according to the Protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
12. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
13. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.

Limitations

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. In immunocom promised patients and newborns serological data only have restricted value.

REFERENCES

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