Mouse Adenovirus-FL and K87 EcoELISA Kit

Cat. No.:DEIA2094
Pkg.Size:96T

Intended use

This kit is to be used for the Detection of Viral/Mycosomal Infections in Laboratory Animals.

General Description

Adenoviruses are medium-sized (90–100 nm), nonenveloped (without an outer lipid bilayer) icosahedral viruses composed of a nucleocapsid and 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 qualitative immunoenzymatic determination of antibodies to Adenoviruses is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiter strip wells are precoated with antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) conjugate is added. This conjugate binds to the captured Adenoviruses antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of antibodies in the specimen. Sulphuric acid is added to stop the reaction. Absorbance at 450 nm is read using an ELISA microwell plate reader.

Reagents And Materials Provided

1. Microtiter Strips: Containing 6 positive and 6 negative antigen coated strips (alternating + [AG] and –[TC] antigen strips): 2 strip holder
2. Dilution Buffer: 25 mL, ready to use. 2 bottle
3. HRP Conjugate: 11 mL, ready to use. 2 vial
4. Positive Control Conjugate: 11 mL, ready to use. 1 vial
5. Substrate Solution: 14 mL, ready to use. Contains Tetramethylbenzidine (TMB). 2 vial
6. Stop Solution: 12 mL, ready to use. Contains 0.5M H_2SO_4, Avoid contact with the stop solution. It may cause skin irritations and burns. 2 vial
7. Wash Solution 40X: 30 mL, concentrate; 1 bottle
8. Positve Control Serum: 0.5 mL. 1 vial
9. Negative Control Serum: 0.1 mL. 1 vial

Materials Required But Not Supplied

1. Calibrated variable precision micropipettes.
2. Absorbent paper.
3. Distilled or deionized water
4. Timer
5. A microtiter plate calibrated reader (450 ± 10 nm)

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Storage

Upon receipt store entire microwell plate and remnant removawell-strips at – 20 ℃ or below.

Reagent Preparation

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

Microtiter Strips

The ready-to-use microwell plate is adsorption coated with partially-purified antigen and tissue control (a solution of bovine serum albumin at the same protein concentration as the antigen), resp. All wells in rows A, C, E, and G are coated with antigen; wells in rows B, D, F, and H are coated with tissue control.

Controls

The sera are supplied diluted in phosphate-buffered saline (PBS) supplemented with 5% w/v bovine serum and 50 µg/mL of gentamycin as a preservative; they have not been heat-inactivated and are non-sterile.If the volume supplied will not be used at one time, divide it into small aliquots and store at - 20 ℃ or below until needed. Frozen antiserum should retain activity for at least 6 months. Once thawed, store at 4 ℃ for no more than 24 hours.

Wash Solution

Add deionized water to the 40X concentrated Wash Solution. Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL. The diluted Wash Solution is stable for 2 weeks at room temperature.

Assay Steps

Dilute test sera 1: 60 with Dilution Buffer. 100 µL per diluted sample is required. Depending on the individual quality of test sera it is recommended to further dilute sample to 1:120 and/or preabsorb unspecific binding moieties by Kaolin pretreatment. Additionaly It is supposed useful to pretreat microwells with 5 % w/v non-fat dry milk in PBS, followed by a wash cycle, prior to adding samples.

1. Add 50 µL of each diluted sample to 2 wells of the antigen-coated microwell plate. One well (A1, C1,...) is the antigen test (AG), the other (B1, D1,...) is the tissue control (TC).
2. Add 50 µL of the diluted controls to the wells. 2 wells per control antiserum (positive and negative; 4 wells).
3. Incubate the filled micro-well-plate at room temperature (RT) for 30 minutes.
4. Fill each well with diluted wash solution, tip plate to remove wash solution quickly (rapid rinse). Repeat 3 times.
5. Fill each plate with wash solution and remove it after 3 minutes (slow rinse). Repeat 3 times.
6. Alternatively use an ELISA Washer – 1.5 mL/well (5 x 300 µL/well).
7. Add 100 µL of HRP Conjugate to all wells.
8. Incubate microwell plate at room temperature (RT) for 30 minutes.
9. Repeat wash procedure, as in step 5, to remove unbound conjugate.
10. Add 100 µL of Substrate Solution to all wells.
11. Incubate at room temperature under observation (on a white background) until the negative and/or diluent control wells content starts developing a distinct blueish colour reaction, but not longer than for 20 minutes.
11. Stop the enzymatic reaction by adding 50 µL of Stop Solution to each well.
12. Read results visually or determine the absorbance (OD) of each well at 450 ± 10 nm with a microtiter plate reader.

Interpretation of Results

The absorbance value (AG-TC) is divided by 0.15 to yield a score.

1. A score less than 1 is negative.
2. A score between 1 and 3 is equivocal and the results must be rechecked.
3. A score more than 3 or equals to 3 is positive.

High reading in the TC wells invalidates the test and the respective sample should be repeated by an alternate assay.

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Precautions

1. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
2. Avoid contact with Stop Solution containing 0.5 mol/L H_2SO_4. It may cause skin irritation and burns.
3. Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
4. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
5. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
6. Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
7. Do not use reagents beyond expiry date as shown on the kit labels.
8. 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.
9. 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.

Limitations

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

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