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User's Manual

Adenovirus (Fecal) ELISA Kit

REF **DEIA2435**

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.

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PRODUCT INFORMATION

Intended Use

This microplate-based ELISA (enzyme linked immunosorbent assay) kit is intended for the qualitative detection of Adenovirus antigen in feces. The assay is a useful tool in the diagnosis of active Adenovirus infection in acute or chronic gastroenteritis.

General Description

Acute diarrheal disease in young children is a major cause of morbidity worldwide and is a leading cause of mortality in developing countries. Research has shown that enteric adenoviruses, primarily Ad40 and Ad41, are a leading cause of diarrhea in many of these children, second only to the rotaviruses. However many different symptoms can manifest, depending on the type of infecting Adenovirus. There are 49 distinct serotypes that can cause infections in humans.

The diarrhea resulting from enteric adenoviruses is longer in duration than that caused by the rotaviruses, usually lasting 7 - 8 days. Adenovirus infections often show up as conjunctivitis, tonsillitis (which may look exactly like strep throat and cannot be distinguished from strep except by throat culture), an ear infection, or croup. Adenoviruses can also cause gastroenteritis (stomach flu). A combination of conjunctivitis and tonsillitis is particularly common with adenovirus infections. Small children are especially prone to develop adenovirus bronchiolitis or pneumonia, both of which can be severe. In babies, adenoviruses can also cause coughing fits that are almost exactly like whooping cough. Adenoviruses can also lead to viral meningitis or encephalitis. Rarely, adenovirus causes inflammation of the urinary bladder (also known as cystitis), producing blood in the urine. In children, adenoviruses may cause acute upper respiratory infections with fever and runny nose. Adenovirus types 1, 2, 3, 5, and 6 are responsible for most of these infections.

Specific diagnosis of the Adenovirus infection is made by identification of the virus in the patient's stool. Enzyme linked immunosorbent assay (ELISA) is the test most widely used to screen clinical specimens.

Principles of Testing

This ELISA is designed, developed and produced for the qualitative measurement of Adenovirus antigen in test specimen. The assay utilizes the microplate-based enzyme immunoassay technique by coating highly purified antibody onto the wall of microtiter well.

Assay controls and fecal specimen, as well as HRP-conjugated monoclonal antibody that specifically recognizes the inner capsid protein of the Adenoviruses are added to microtiter wells of microplate that was coated with a highly purified polyclonal anti Adenovirus antibody on its wall. After an incubation period an immunocomplex of "Anti-Adenovirus Antibody - Adenovirus Antigen - HRP-conjugated Anti-Adenovirus Tracer Antibody" is formed if there is Adenovirus antigen present in the test sample. The unbound tracer antibody and other protein or buffer matrix are removed in the subsequent washing step. HRP-conjugated tracer antibody bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the tracer antibody bound to Adenovirus captured on the wall of each microtiter well is directly proportional to the amount of Adenovirus antigen level in each test specimen.

Reagents And Materials Provided

Prior to use allow all reagents to come to room temperature.

1. **Anti-Adenovirus Antibody Coated Microplate:** One microplate with 12 x eight strips (96 wells total) coated with highly purified anti-Adenovirus antibody. The plate is framed and sealed in a foil Ziploc bag with a desiccant. This reagent should be stored at 2 - 8°C and is stable until the expiration date on the kit box.
2. **Anti-Adenovirus Tracer Antibody:** One vial containing 0.6 mL concentrated horseradish peroxidase (HRP)-conjugated monoclonal anti-Adenovirus tracer antibody in a stabilized protein matrix. This reagent must be diluted with Tracer Antibody Diluent before use. This reagent should be stored at 2 - 8°C and is stable until the expiration date on the kit box.
3. **Tracer Antibody Diluent:** One vial containing 12 mL ready-to-use buffer. It should be only used for antibody dilution according to the assay procedures. This reagent should be stored at 2 - 8°C and is stable until the expiration date on the kit box.
4. **ELISA Wash Concentrate:** One bottle contains 30 mL of 30 fold concentrate. Before use the contents must be diluted with 870 mL of demineralized water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate-buffered saline with a non-azide preservative. The diluted wash buffer should be stored at room temperature and is stable until the expiration date on the kit box.
5. **ELISA HRP Substrate:** One bottle contains 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 - 8°C and is stable until the expiration date on the kit box.
6. **ELISA Stop Solution:** One bottle contains 12 mL of 0.5 M sulfuric acid. This reagent should be stored at 2 - 8°C or room temperature and is stable until the expiration date on the kit box.
7. **Adenovirus Antigen Controls:** One vial contains Adenovirus negative control and another vial contains inactivated Adenovirus positive control. Both controls are in a liquid bovine serum albumin-based matrix with a non-azide preservative. **Refer to vials for exact concentration range for each control.** After the first use, the calibrators should be stored at -20°C or below for long-term storage.
8. **Concentrated Patient Sample Diluent:** One bottle contains 30 mL of 20-fold concentrated buffer matrix with protein stabilizers and preservative. This reagent should be stored at 2 - 8°C and is stable until the expiration date on the kit box. Before use the concentrated buffer must be diluted with 570 mL of distilled water and mixed well. Upon dilution this yields a working patient sample diluent containing a surfactant in phosphate buffered saline with a non-azide preservative. The diluted sample diluent can be stored at room temperature and is stable for 8 weeks. It can also be stored at 2 - 8°C and is stable until the expiration date on the kit box.

Materials Required But Not Supplied

1. Precision single channel pipettes capable of delivering 10 µL, 50 µL, 100 µL, and 1000 µL, etc.
2. 25 - 50 µL inoculating loop.
3. Repeating dispenser suitable for delivering 100 µL.
4. Disposable pipette tips suitable for above volume dispensing.
5. Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes.
6. Disposable plastic 1000 mL bottle with caps.
7. Aluminum foil.
8. Deionized or distilled water.
9. Plastic microtiter well cover or polyethylene film.

10. ELISA multichannel wash bottle or automatic (semiautomatic) washing system.
11. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

Storage

This test kit must be stored at 2 - 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Specimen Collection And Preparation

1. Stool specimens can be collected at any time of the day.
2. Collect a random sample of feces into a fecal sample collection container or tube or cup with an aid of a clean, dry cup or plastic spoon or toilet paper.
3. It is required to collect minimum 0.1 mL liquid stool sample or 0.1 g solid sample.
4. The specimen is ready for testing, transportation or storage. It can be stored at 2-8°C for up to 3 days and at frozen condition (-20°C) for longer storage.

Patient Sample Preparation

Patient sample needs to be diluted 1:11 with patient sample diluent working solution before being measured.

1. Label a test tube (12 x 75 mm) or a 1.5 ml plastic vial.
2. Add 1 mL of the diluted Patient Sample Diluent to each tube or vial.
3. Add 100 µL of liquid stool sample to the above tube.
4. With solid stool sample, take an equivalent amount (about 50 - 100 mg) with a spatula or a disposable inoculation loop. Suspend the solid stool sample with 1 mL patient sample diluent and mix well in a vortex mixer. Allow the diluted sample to sediment for about 5 minutes. The supernatant can be directly used in the assay.
5. If the test procedure is performed on an automated ELISA system, the supernatant must be particle-free by centrifuging the sample at 5000 rpm (2000 - 2500 g) for 5 minutes.

Reagent Preparation

1. Prior to use allow all reagents to come to room temperature.
2. ELISA Wash Concentrate must be diluted to working solution prior use. Please see Reagents And Materials Provided section for details.
3. Concentrated Patient Sample Diluent must be diluted to working solution prior use. Please see Reagents And Materials Provided section for details.

Assay Procedure

1. Place a sufficient number of anti-Adenovirus antibody coated microwell strips in a holder to run Adenovirus controls and unknown samples in duplicate.
2. Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	Control Negative	SAMPLE 3	SAMPLE 7
B	Control Negative	SAMPLE 3	SAMPLE 7
C	Control Positive	SAMPLE 4	SAMPLE 8
D	Control Positive	SAMPLE 4	SAMPLE 8
E	SAMPLE 1	SAMPLE 5	
F	SAMPLE 1	SAMPLE 5	
G	SAMPLE 2	SAMPLE 6	
H	SAMPLE 2	SAMPLE 6	

3. Prepare working anti-Adenovirus tracer antibody working solution by **1:21 fold** dilution of the Anti-Adenovirus Tracer Antibody with the Tracer Antibody Diluent. For each strip, it is required to mix 0.5 mL of Tracer Antibody Diluent with 25 µL of Tracer Antibody in a clean test tube.
4. Add **100 µL** of controls and diluted patient stool samples into each designated microwell.
5. Add **50 µL** of above diluted tracer antibody working solution to each of the wells.
6. Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
7. Incubate plate at room temperature for **1 hour**.
8. Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
9. Add **100 µL** of ELISA HRP Substrate into each of the wells.
10. Cover the plate with aluminum foil to avoid exposure to light.
11. Incubate plate at room temperature for 10 to 20 minutes.
12. Remove the aluminum foil. Add **100 µL** of ELISA Stop Solution into each of the wells. Mix gently.
13. Read the absorbance at 450 nm within 10 minutes in a microplate reader.

Procedural Notes:

1. It is recommended that all controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original amber bottles.
3. Store any unused antibody coated strips in the foil Ziploc bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results. 6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
7. All reagents should be mixed gently and thoroughly prior use. Avoid foaming.

Quality Control

To assure the validity of the test run, the OD value of the negative control must be below 0.15 and the OD of the positive control must be greater than 0.80. Moreover, each assay should include adequate controls with known Adenovirus antigen level. We recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

Interpretation Of Results

1. Calculate the average absorbance for each pair of duplicate test results.
2. Calculate the cut-off:

   The positive cut-off and the negative cut-off is established by using following formula.

   Positive Cut-Off = 1.1 x (mean extinction of negative control + 0.08)

   Negative Cut-Off = 0.9 x (mean extinction of negative control + 0.06)
3. Interpret test result

   Positive: patient sample extinction is greater than the Positive Cut-Off.

   Negative: patient sample extinction is less than the Negative Cut-Off.

   Equivocal: patient sample extinction is between the Positive Cut-Off and the Negative Cut-Off.

Typical Standard Curve

A typical absorbance data from both negative control and positive control are represented. **This result should not be used in lieu of patient sample test result run with each assay.**

	OD 450 nm		
	Test 1	Test 2	Average
Negative Control	0.118	0.082	0.100
Positive Control	2.845	2.890	2.868

***Negative Cut-Off = 1.1 x (0.1 + 0.08) = 0.198**

***Positive Cut-Off = 0.9 x (0.1 + 0.06) = 0.144**

*Note: *The above Negative and Positive Cut-Offs are for demonstration purposes only and must not be used as routine reference for test result interpretation in clinical laboratory. Every assay should include Negative and Positive Controls to interpret test results of unknown samples.*

Excepted Results

Normal healthy individuals should be free of Adenovirus antigen in feces and should show a negative test result. A positive test result indicates that the patient is shedding detectable amounts of Adenovirus antigen. Incidence of Adenovirus infection varies significantly in populations, season of the year, and geographic regions.

Specificity

This assay does not cross react to the following organisms: Rotavirus, Giardia iamblia, and Cryptosporordium parvum.

Reproducibility

The reproducibility of this assay was validated by measuring two positive samples and one negative sample in 5 different assays run on different days. The results showed a consistent result interpretation for all the

samples.

Precautions

The reagents must be used in laboratory and are for professional use only. The source material for reagents containing bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

Limitations

1. The results obtained with this fecal Adenovirus antigen test kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves. Adenovirus antigen negative results in untreated patients does not rule out infection.
2. Since there is no Gold Standard concentration or controls available for Adenovirus antigen measurement, the values of assay controls were established and calibrated by the kit manufacturer.
3. Large particle of feces in a test sample and being added to microtiter plate would cause unexpected false test results.
4. Water deionized with polyester resins may inactive the horseradish peroxidase.