EPITOPE DIAGNOSTICS, INC.

EDI™ Fecal Adenovirus Antigen ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the Measurement of Adenovirus Antigen in Feces

REF KT-842 CE IVD 96

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. It is for in vitro diagnostic use only.

SUMMARY OF PHYSIOLOGY

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.

ASSAY PRINCIPLE

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 recognize 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: PREPARATION AND 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.

1. Anti-Adenovirus Antibody Coated Microplate (30476)
Microplate coated with an highly purified antibody.
Qty: 1 X 96 well microplate
Storage: 2 – 8°C
Preparation: Ready to Use.

2. Anti-Adenovirus Tracer Antibody (30477)
HRP-conjugated monoclonal anti-adenovirus tracer antibody in a stabilized protein matrix.
Qty: 1 X 0.6 mL
Storage: 2 – 8°C
Preparation: 21X Concentrate. This reagent must be diluted with Tracer Antibody Diluent (30710) before use.

3. Tracer Antibody Diluent (30710)
Buffer for the dilution of the Anti-Adenovirus Tracer Antibody (30477).
Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use.

4. ELISA Wash Concentrate (10010)
A surfactant in phosphate-buffered saline with a non-azide, non-mercury preservative.
Qty: 1 X 30 mL bottle
Storage: 2 – 25°C
Preparation: 30X Concentrate. The contents must be diluted with 870 mL of demineralized water and mixed well before use.

5. ELISA Wash HRP Substrate (10020)
Tetramethylbenzidine (TMB) with stabilized hydrogen perioxide.
Qty: 1 X 12 mL bottle
Storage: 2 – 8°C
Preparation: Ready to Use

6. ELISA Stop Solution (10030)
0.5 M sulfuric acid
Qty: 1 X 12 mL bottle
Storage: 2 - 25°C
Preparation: Ready to Use

7. Adenovirus Antigen Controls (30478, 30479)
Negative (30478) and Positive (30479) controls in a liquid bovine serum albumin-based matrix with a non-azide preservative.
Qty: 2 X vials per control
Storage: 2 – 8°C, After the first use, the calibrators should be stored at -20°C or below for long-term storage. Do not exceed 3 freeze-thaw cycles.
Preparation: Ready to Use

8. Concentrated Patient Sample Diluent (30189)
Concentrated buffer matrix with protein stabilizers and preservatives.
Qty: 1 X 30 mL bottle
Storage: 2 - 8°C
Preparation: 20X Concentrate. The contents must be diluted with 570 mL of demineralized water and mixed well before use.

SAFETY PRECAUTIONS

The reagents are for in vitro diagnostic 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.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 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 (semi-automatic) washing system.
11. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

SPECIMEN COLLECTION & STORAGE

1. Stool specimens can be collected at any time of the day.
2. Collect sample with a fecal sample collection container.
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 ºC for up to 3 days and at frozen condition (-20ºC) for longer storage.

ASSAY PROCEDURE

1. Reagent Preparation

1. Prior to use allow all reagents to come up to room temperature (20-25 ºC). Reagents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate (10010) must be diluted to working solution prior use. Please see REAGENTS section for details.
3. Concentrated Patient Sample Diluent (30189) must be diluted to working solution prior use. Please see REAGENTS section for details.

2. Patient Sample Preparation

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

1. Label a test tube (12x75 mm) or a 1.5 mL plastic vial.
2. Add **1 mL** of the diluted Patient Sample Diluent (30189) 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**.

3. Assay Procedure

1. Place a sufficient number of Adenovirus Antibody Coated microwell strips (30476) 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. Add **100 µL** of the controls (30478,30479) and diluted patient stool samples into each designated microwell.
4. Cover the plate with one plate sealer and also with aluminum foil. Incubate plate at **room temperature (20-25 ºC)** for **60 minutes**.
5. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
1. Prepare the antibody working solution by 1:21 fold dilution of the antibody (30477) with the diluent (30710) in a clean test tube. For each strip, it is required to mix 1 mL of the diluent (30710) with 50 µL of the antibody (30477) in a clean test tube.

   Note: This antibody working solution should be freshly prepared.
6.
7. Add **100 µL** of the antibody working solution to each of the wells.
8. Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
9. Incubate plate at **room temperature (20-25 ºC)** for **30 minutes**.
10. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
11. Add **100 µL** of ELISA HRP Substrate (10020) into each of the wells.
12. Cover the plate with aluminum foil to avoid exposure to light.
13. Incubate plate at **room temperature (20-25 ºC)** for **20 minutes**.
14. Remove the aluminum foil. Add **100 µL** of ELISA Stop Solution (10030) into each of the wells. Mix gently.
15. 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.

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 the 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.

EXAMPLE DATA AND CALCULATION

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	0.078	0.080	0.079
Positive Control	1.865	1.774	1.820

***Negative Cut-Off = 0.9 x (0.079 + 0.06) = 0.125 *Positive Cut-Off = 1.1 x (0.079 + 0.08) = 0.175**

*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.*

EXPECTED 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.

LIMITATION OF THE PROCEDURE

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.

PERFORMANCE CHARACTERISTICS

Reproducibility

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

Specificity

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

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.

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

REFERENCES

1. Edwards KM, Thompson J, Paolini J, Wright PF. Adenovirus infections in young children. *Pediatrics.* 1985 Sep;76(3):420-424
2. Halonen P, Sarkkinen H, Artstila P, Hjertsson E, Torfason E. Four-layer radioimmunoassay for detection of adenovirus in stool. *J Clin Microbiol*. 1980 jun;11(6):614-617.
3. Meurman O, Ruuskanen O, Sarkkinen H. Immunoassay diagnosis of adenovirus infections in children. *J Clin Microbiol.* 1983 Nov;18(5):1190-1195

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.

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Please visit our website at www.epitopediagnostics.com to learn more about our products and services.

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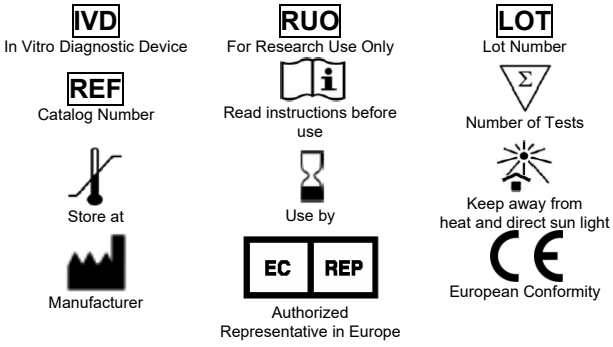
GLOSSARY OF SYMBOLS (EN 980/ISO 15223)



SHORT ASSAY PROCEDURE

1. Add **100 µL** of the controls and samples into the designated microwells.
2. Add **100 µL** of the assay buffer to each well.
3. Mix, cover, and incubate at **room temperature (20-25 ºC)** for **60 minutes**.
4. Wash each well five times.
5. Add **100 µL** of the Antibody Working Solution to each well.
6. Cover and incubate at **room temperature (20-25 ºC)** for **30 minutes**.
7. Wash each well five times
8. Add **100 µL** of substrate to each well.
9. Cover and incubate at **room temperature (20-25 ºC)** for **20 minutes**.
10. Add **100 µL** of the stop solution to each well.
11. Read the absorbance at **450 nm**.