Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
fax 02-92157285
info@listarfish.it
www.listarfish.it

EDI™ Fecal Rotavirus Antigen ELISA Kit

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

REF **KT 841** EU: IVD CE Σ **96**

INTENDED USE

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

SUMMARY OF PHYSIOLOGY

Rotaviruses are the main and the most important pathogens that cause non-bacterial acute gastroenteritis and diarrhea, especially in children from 6 months to 2 years of age, premature infants, the elderly, and immunocompromised individuals. Rotaviruses have been identified in almost 40% of the faces of children with gastroenteritis. Rotavirus is the cause of up to 50% of the hospitalized cases of diarrhea in infant and young children. Almost every child has been infected with rotavirus by age 5. Over 3 million cases of rotavirus gastroenteritis occur annually in the U.S.. There are about 120 million rotavirus infections every year worldwide and that causes the death of 600,000 to 650,000 children. Study also indicates that a high frequency of rotavirus infections may increase the risk of celiac disease autoimmunity in children with genetic predisposition individuals.

Rotaviruses have a genome consisting of 11 double-stranded RNA segments surrounded by a distinctive three-layered icosahedral protein capsid. The first layer is formed by the protein VP2, with each vertex having a copy of the proteins VP1 and VP3. The second layer is formed by the protein VP6. The outermost protein layer is composed of the structural glycoprotein VP7 and the spike protein VP4. Viral particles are up to 100 nm in diameter and have a buoyant density of 1.36 g/ml in CsCl. Rotaviruses tend to affect gastrointestinal epithelial cells that are at the tip of the villus. Their triple protein coats make them very resistant to the normally prohibitive pH of the stomach, and also digestive enzymes (lipases and proteases) in the gastrointestinal tract. During the infection, rotavirus produces mRNA to support both protein translation and genome replication.

Rotavirus is transmitted by oral-fecal contact with an incubation period of 1-3 days. Characteristic symptoms include vomiting, hydrodiarrhoea for between 3 and 8 days, high temperature and stomach pains. A large amount of rotavirus particles is shed during infection.

Specific diagnosis of the rotavirus infection is made by identification of the virus in the patient's stool. Enzyme linked immunsorbent assay (ELISA) is the test most widely used to screen clinical specimens. Electron microscopy and polyacrylamide gel electrophoresis are used in some laboratories in addition or as an alternative to ELISA.

ASSAY PRINCIPLE

This ELISA is designed, developed and produced for the qualitative measurement of rotavirus 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 specifically recognize the inner capsid protein of the rotaviruses are added to microtiter wells of microplate that was coated with a highly purified polyclonal anti-rotavirus antibody on its wall. After an incubation period an immunocomplex of "Anti-Rotavirus Antibody – Rotavirus Antigen – HRP-conjugated Anti-rotavirus Tracer Antibody" was formed if there is rotavirus 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 rotavirus captured on the wall of each microtiter well is directly proportional to the amount of rotavirus 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.

Prior to use allow all reagents to come to room temperature.
Reagents from different kit lot numbers should not be combined or interchanged.

1. **Anti-Rotavirus Antibody Coated Microplate (Cat. No. 30185)**
   One microplate with 12 x eight strips (96 wells total) coated with highly purified anti-rotavirus 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-Rotavirus Tracer Antibody (Cat. No. 30186)**
   One vial containing 0.6 mL concentrated horseradish peroxidase (HRP)-conjugated monoclonal anti-rotavirus 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 (Cat. No. 30052)**
   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 (Cat. No. 10010)**
   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 (Cat. No. 10020)
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 (Cat. No. 10030)
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. Rotavirus Antigen Controls (Cat. No. 30187 – 30188)
One vial contains rotavirus negative control (30187) and another vial contains inactivated rotavirus positive control (30188). 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 (Cat. No. 30189)
One bottle contains 30 mL of 10-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 290 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.

SAFETY 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.

MATERIALS REQUIRED BUT NOT PROVIDED

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 (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 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 2-8ºC 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 to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
(2) ELISA Wash Concentrate (Cat. 10010) must be diluted to working solution prior use. Please see REAGENTS section for details.
(3) Concentrated Patient Sample Diluent (Cat. 30189) must be diluted to working solution prior use. Please see REAGENTS section for details.

2. 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 (12x75 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.

3. Assay Procedure

(1) Place a sufficient number of anti-rotavirus antibody coated microwell strips (Cat. 30185) in a holder to run rotavirus 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-rotavirus tracer antibody working solution by **1:21 fold** dilution of the Anti-Rotavirus Tracer Antibody (Cat. 30186) with the Tracer Antibody Diluent (Cat. 30052). 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 (Cat. 30187-30188)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 (Cat. 10020) 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 (Cat. 10030) 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 mix 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 absorbance of negative control + 0.08)
   Negative Cut-Off = 0.9 x (mean absorbance of negative control + 0.06)

3. Interpret test result
   - **Positive:** patient sample absorbance is greater than the Positive Cut-Off.
   - **Negative:** patient sample absorbance is less than the Negative Cut-Off.
   - **Equivocal**: patient sample absorbance is between the Positive Cut-Off and the Negative Cut-Off.

EXAMPLE DATA AND CALIBRATOR 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.**

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

EXPECTED RESULTS

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

LIMITATION OF THE PROCEDURE

1. The results obtained with this fecal rotavirus antigen test kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves. Rotavirus antigen negative results in untreated patients does not rule out the infection.
2. Since there is no Gold Standard concentration or controls available for rotavirus 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 enzyme.

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 rotavirus antigen level. We recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

PERFORMANCE CHARACTERISTICS

Reproducibility

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

Specificity

The assay doesn't cross react to following organisms: Adenovirus, Giardia lamblia, Cryptosporidium parvum.

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

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This product is developed and manufactured by

Epitope Diagnostics, Inc.
San Diego, CA 92121, USA

CE EC REP

MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

Manufacturer	No. of tests
REF Catalog Number	Keep away from heat and direct sun light
CONC Concentrate	Store at
IVD In Vitro Diagnostic Device	Use by
Read instructions before use	LOT Lot No.
EC REP Authorized Representative In Europe	

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