Package Insert

Fecal Rotavirus Antigen ELISA Assay Kit

Catalog Number:

RTV35-K01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

v. 7.0 (*effective 06Jun23)*

Eagle Biosciences, Inc.

20A Northwest Blvd., Suite 112, Nashua, NH 03063

Phone: 617-419-2019 Fax: 617-419-1110

www.EagleBio.com

INTENDED USE

Eagle Biosciences Fecal Rotavirus Antigen ELISA (enzyme linked immunosorbent assay) kit is intended for the qualitative and quantitative detection of rotavirus antigen in feces. Specifically, this test detects VP6 of the viral capsid and is useful in detection of type A rotavirus infection. The Eagle Biosciences Fecal Rotavirus Antigen ELISA Assay kit is intended for research use only and not intended for diagnostic procedures.

ASSAY BACKGROUND

Rotaviruses are the main and the most important pathogens that cause of non-bacterial acute gastroenteritis and diarrhea, especially in children from 6 months to 2 years of age, premature infants, the elderly, and the 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 US. 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 childhood in genetically predisposed 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 immunosorbent 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.

PRINCIPLE OF THE ASSAY

This Eagle Biosciences Fecal Rotavirus Antigen ELISA is designed, developed and produced for the qualitative and quantitative 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 that 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 Eagle Biosciences Fecal Rotavirus Antigen ELISA Assay 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-Rotavirus Antibody Coated Microplate

Microplate coated with a highly purified anti-rotavirus antibody.

Qty: 1 x 96 well microplate
Storage: 2 – 8 °C
Preparation: Ready to Use

2. Anti-Rotavirus Tracer Antibody

HRP-conjugated monoclonal anti-rotavirus tracer antibody in a stabilized protein matrix

Qty: 1 x 12 mL
Storage: 2 – 8 °C
Preparation: Ready to Use

3. ELISA Wash Concentrate

Surfactant in a phosphate buffered saline with non-azide preservative.

Qty: 1 x 30 mL
Storage: 2 – 25 °C
Preparation: 30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.

4. ELISA HRP Substrate

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.

Qty: 1 x 12 mL
Storage: 2 – 8 °C
Preparation: Ready to Use

5. ELISA Stop Solution

0.5 M sulfuric acid.

Qty: 1 x 12 mL
Storage: 2 – 25 °C
Preparation: Ready to Use

6. Rotavirus Antigen Calibrator 6

1.5 mL of a calibrator in bovine serum albumin-based matrix with a non-azide preservative.

Qty:	1 x vial
Storage:	2 – 8 °C
Preparation:	Ready to Use

7. Concentrated Patient Sample Diluent

Concentrated buffer matrix with protein stabilizers and preservative. Upon dilution this yields a working calibrator 6 matrix buffer, negative control, and 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.

Qty:	30 mL
Storage:	2 – 8 °C
Preparation:	10X Concentrate. The contents must be diluted with 270 mL distilled water and mixed well before use.

SAFETY PRECAUTIONS

The reagents are for research use only. Source material which contains reagents of bovine serum albumin 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 hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. 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

- Precision single channel pipettes capable of delivering 10 µL, 25 µL, 50 µL, 65 µL, 100 µL, and 1000 µL, etc.
- Repeating dispenser suitable for delivering 100 µL.
- Disposable pipette tips suitable for above volume dispensing.
- Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes.
- Disposable plastic 1000 mL bottle with caps.
- Aluminum foil.
- Plastic microtiter well cover or polyethylene film.
- ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
- 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 (20-25 ºC). Reagents from different kit lot numbers should not be combined or interchanged.

(2) ELISA Wash Concentrate must be diluted to working solution prior use. Please see REAGENTS section for details.

(3) Concentrated Patient Sample Diluent must be diluted to working solution prior use. Please see REAGENTS section for details.

(4) Prepare 1:3 serially diluted calibrators using Rotavirus Ag Calibrator Level 6 and 1x Patient Sample Diluent as the dilution buffer. Store at 2-8ºC, -20ºC for long term storage, Avoid more than 3x freeze thaw cycle.

Calibrator	Volume of Calibrator (mL)	Volume of 1x Patient Sample Diluent (mL)
Level 6	1.5 mL Level 6	-
Level 5	0.5 mL Level 6	1 mL
Level 4	0.5 mL Level 5	1 mL
Level 3	0.5 mL Level 4	1 mL
Level 2	0.5 mL Level 3	1 mL
Level 1	-	1.5 mL

2. Specimen Preparation

2.1. Manual Weighing:

Specimen need to be diluted **1:11** with 1x Patient Sample Diluent before being measured.

(1) Label a test tube (12x75 mm) or a 4 ml plastic vial.

(2) With solid stool sample, take or weigh an equivalent amount (about **90mg,** size of a green pea) with a spatula or a disposable inoculation loop. Suspend the solid stool sample with **1 mL 1x Patient Sample Diluent** and mix well on a vortex mixer.

(3) Centrifuge the diluted fecal sample at 3000 rpm (800- 1500 g) for 5-10 minutes. The supernatant can be directly used in the assay. As an alternative to centrifuging, let the diluted samples sit and sediment for 30 minutes and take the clear supernatant for testing. Note: If the test procedure is performed on an automated ELISA system, the supernatant must be particle-free by centrifuging the sample.

(4) This sample can be stored at 2-8ºC up to three (3) days and below -20ºC for longer storage. Avoid more than 3x freeze and thaw cycle.

2.2. Using Fecal Sample Collection Devices

(1) Label a Fecal Sample Collection tube

(2) Follow the instructions on the Sample Collection Tube insert.

(3) This sample can be stored at 2-8ºC up to three (3) days and below -20ºC for longer storage. Avoid more than 3x freeze and thaw cycle.

(4) Two drops of the extracted sample is equivalent to 100 µl.

3. Qualitative Assay Procedure

(1) Place a sufficient number of microwell strips in a holder to run diluted calibrators and diluted samples in duplicate.

(2) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	CAL 1	CAL 5	SAMPLE 3
B	CAL 1	CAL 5	SAMPLE 3
C	CAL 2	CAL 6	SAMPLE 4
D	CAL 2	CAL 6	SAMPLE 4
E	CAL 3	SAMPLE 1	SAMPLE 5
F	CAL 3	SAMPLE 1	SAMPLE 5
G	CAL 4	SAMPLE 2	SAMPLE 6
H	CAL 4	SAMPLE 2	SAMPLE 6

(3) Add **100 µL** of diluted calibrators and diluted samples into the designated microwell. Mix gently by tapping the plate. ***Note:*** *if the collection tubes are used, add two drops of extracted fecal sample into each well.*

(4) Cover the plate with one plate sealer and aluminum foil. Incubate plate at room temperature (20-25ºC) for **60 minutes.**

(5) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL diluted wash solution into each well and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.

(6) Add **100 µL** of above tracer antibody solution to each of the wells. Mix by gently tapping the plate.

(7) Cover the plate with one plate sealer and aluminum foil. Incubate plate at room temperature (20-25 ºC) for **30 min**.

(8) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of diluted wash solution into each well and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.

(9) Add **100 µL** of ELISA HRP Substrate into each of the wells. Mix gently by tapping the plate.

(10) Cover the plate with one plate sealer and aluminum foil. Incubate plate at room temperature (20-25 ºC) for 20 minutes.

(11) Remove the aluminum foil. Add **100 µL** of ELISA Stop Solution into each of the wells. Mix gently by tapping the plate.

(12) Read the absorbance at 450 nm within 10 minutes in a microplate reader.

4. Quantitative Assay Procedure

(1) Place a sufficient number of microwell strips in a holder to run positive control (Calibrator Level 6), negative control (diluted 1X patient sample diluent), and diluted samples in duplicate.

(2) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	NEG CTRL	SAMPLE 3	SAMPLE 7
B	NEG CTRL	SAMPLE 3	SAMPLE 7

C	POS CTRL	SAMPLE 4	SAMPLE 8
D	POS CTRL	SAMPLE 4	SAMPLE 8
E	SAMPLE 1	SAMPLE 5	SAMPLE 9
F	SAMPLE 1	SAMPLE 5	SAMPLE 9
G	SAMPLE 2	SAMPLE 6	SAMPLE 10
H	SAMPLE 2	SAMPLE 6	SAMPLE 10

(3) Add **100 µL** of positive control (Calibrator Level 6), negative control (diluted 1X patient sample diluent), and diluted samples into the designated microwells. Mix by gently tapping the plate. ***Note:*** *if the collection tubes are used, add two drops of extracted fecal sample into each well.*

(4) Cover the plate with one plate sealer and aluminum foil. Incubate plate at room temperature (20-25ºC) for **60 minutes.**

(5) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL diluted wash solution into each well and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.

(6) Add **100 µL** of above tracer antibody solution to each of the wells. Mix by gently tapping the plate.

(7) Cover the plate with one plate sealer and aluminum foil. Incubate plate at room temperature (20-25 ºC) for **30 min**.

(8) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of diluted wash solution into each well and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.

(9) Add **100 µL** of ELISA HRP Substrate into each of the wells. Mix gently by tapping the plate.

(10) Cover the plate with one plate sealer and aluminum foil. Incubate plate at room temperature (20-25 ºC) for 20 minutes.

(11) Remove the aluminum foil. Add **100 µL** of ELISA Stop Solution into each of the wells. Mix gently by tapping the plate.

(12) 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. Store any unused antibody-coated strips in the foil Ziploc bag with desiccant to protect from moisture. Exposure of the plates to humidity drastically reduces the shelf life.
3. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
4. Incubation times or temperatures other than those stated in this insert may affect the results.
5. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
6. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

INTERPRETATION OF RESULTS

Quantitative Measurement

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the calibrator 1 (0 U/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The calibration curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results.
4. The Rotavirus concentrations for the unknown samples are read directly from the calibrator curve using their respective corrected absorbance.

Qualitative Measurement

1. Positive or reactive: Any sample well that is obviously more yellow than the negative control well.
2. Negative or non-reactive: Any sample well that is not obviously more yellow than the negative control well.

Note: The negative control, as well as some patient samples, may show some slight yellow color. A sample well must be obviously darker or more yellow than the negative control well, when it is interpreted as a positive result.

ELISA Reader:

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 are 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.

4. Assay quality control

    1. Positive control must show an average OD reading greater than 0.8.
    2. Negative control should show an average OD reading less than 0.1.

LIMITATIONS OF THE PROCEDURE

1. Positive: patient sample extinction is greater than the Positive Cut-Off
2. Negative: Patient sample extinction is less than the Negative Cut-off

3. Equivocal: Patient sample extinction is between the Positive Cut-off and the Negative Cut-off

QUALITY CONTROL

To assure the validity of the test run, the OD value of the negative control must be below 0.1 and the OD of the positive control must be greater than 0.8. 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 Fecal Rotavirus Antigen ELISA Assay Kit.

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.

Stool from 40 normal adults were measured with this ELISA. We found that normal people show undetectable Rotavirus antigen in the extracted stool sample according to the sample collection, extraction and assay procedures described in this insert. The suggested positive cut-off for fecal Rotavirus antigen is 3 U/mL.

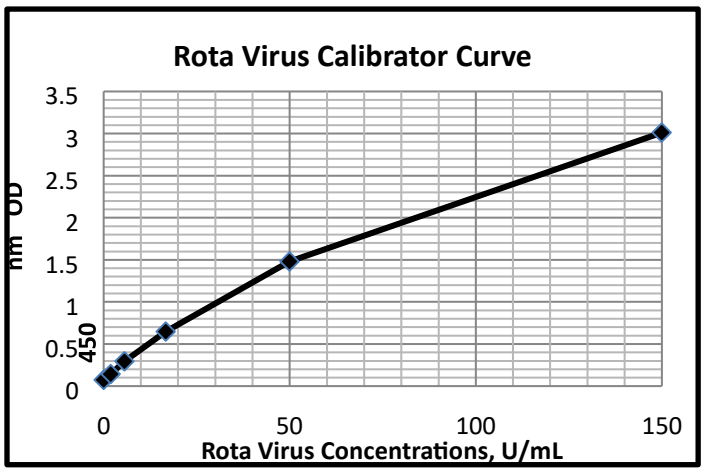
EXAMPLE DATA

1. Quantitative Measurement:

A typical absorbance data and the resulting calibration curve from Fecal Rotavirus Antigen ELISA represented.

Note: This curve should not be used in lieu of calibration curve run with each assay.

Well I.D.	Reading Absorbance (450 nm)	
	Average	Corrected
Calibrator Level 1: 0 U/mL	0.074	0
Calibrator Level 2: 1.9 U/mL	0.144	0.07
Calibrator Level 3: 5.6 U/mL	0.296	0.222
Calibrator Level 4: 16.7 U/mL	0.647	0.573
Calibrator Level 5: 50.0 U/mL	1.481	1.407
Calibrator Level 6: 150.0 U/mL	3.011	2.937



Qualitative Measurement:

	Average OD 450 nm
Negative Control	0.074
Positive Control	3.011

Positive Cut-Off = 1.1 x (0.074 + 0.08) = 0.1694
Negative Cut-Off = 0.9 x (0.074 + 0.06) = 0.1206
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REFERENCES

1. Set-up of a new rapid immunochromatographic diagnostic test for a Rotavirus detection. D. Van Beers , M. DE Foor , R. Viehoff , D. Col , M. Venuti and T. Leclipteux.Progress in Clinical Virology III , Bologne , Septembre 1997.
2. Detection of rotavirus in faecal specimens with a monoclonal antibody enzyme-linked immunosorbent assay: comparison with polyclonal antibody enzyme-immunoessays and a latex agglutination test. Sneyers et al.Comp. Immun. Microbiol. Infect. Dis. , vol 12 , n°4 , pp 95104 , 1989
3. Comparison of Three Rapid Immunoassays for the Detectiono of Rotavirus Antigen in Stool Samples I. Van der Donck et al. ESCV Winter Meeting 1999, Rotterdam, the Netherlands
4. Evaluacion de tres Metodos de Deteccion de Rotavirus en Heces I. Wilhelmi et al. 6th Congresso Nacional de Virologia, Madrid, 26th Oct. 99

SHORT ASSAY PROCEDURE

1. Quantitative Measurement
    1. Add 100 µL of the diluted calibrators and diluted samples or two drops of Specimen into the designated microwells.
    2. Mix, cover, and incubate at room temperature (20-25 °C) for 60 minutes.
    3. Wash each well five times.
    4. Add 100 µL of the tracer antibody to each well.
    5. Cover and incubate at room temperature (20-25 °C) for 30 minutes.
    6. Wash each well five times
    7. Add 100 µL of substrate to each well.
    8. Cover and incubate at room temperature (20-25 °C) for 20 minutes.
    9. Add 100 µL of the stop solution to each well.
    10. Read the absorbance at 450nm.
2. Qualitative Measurement
    1. Add 100 µL of of positive control [Calibrator level 6], negative control [diluted 1X patient sample diluent], and diluted samples or two drops of Specimen into the designated microwells.
    2. Mix, cover, and incubate at room temperature (20-25 °C) for 60 minutes.
    3. Wash each well five times.
    4. Add 100 µL of the tracer antibody to each well.
    5. Cover and incubate at room temperature (20 25 °C) for 30 minutes.
    6. Wash each well five times
    7. Add 100 µL of substrate to each well .
    8. Cover and incubate at room temperature (20-25 °C) for 20 minutes.
    9. Add 100 µL of the stop solution to each well.
    10. Read the absorbance at 450nm.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.