EPITOPE DIAGNOSTICS, INC.

EDI™ Fecal Cryptosporidium parvum Antigen ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the Detection of Cryptosporidium parvum Antigen in Feces

REF KT-839 CE IVD 96

INTENDED USE

This microplate-based ELISA (enzyme linked immunosorbent assay) kit is intended for the qualitative detection of *Cryptosporidium parvum* antigen in feces. The assay is a useful tool in the diagnosis of active *Cryptosporidium parvum* infection in acute or chronic diarrhea. This kit is for in vitro diagnostic use only.

SUMMARY OF PHYSIOLOGY

Cryptosporidiosis is one of the main causes of persistent diarrhea in the developed world. It is caused by the presence of Cryptosporidium parvum oocysts in the gastro-intestinal tract. This parasite is known to be highly pathogenic and its infectious stage is transmitted by faecal-oral contract. It is also an opportunistic pathogen found in immunocompromised patients.

The symptoms of cryptosporidiosis are watery diarrhea, stomach cramps, weight loss, nausea, and fever[1]. In industrialized countries, 2-2.5% of diarrhreal hospitalized patients shed C. parvum oocysts. Ten percent of AIDS patients have chronic cryptosporidiosis and this figure can be as high as 40% in certain developing countries.

C. parvum is diagnosed by either Ziehl-Neelsen stain or immunofluorescence in smears of unconcentrated specimens.

ASSAY PRINCIPLE

This "sandwich" ELISA is designed, developed and produced for the qualitative measurement of *Cryptosporidium parvum* antigen in stool 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 are added to microtiter wells of microplate that was coated with a highly purified polyclonal anti-*Cryptosporidium parvum* antibody on its wall. The *Cryptosporidium parvum* antigen will be bound to the antibody coated plate after an incubation period. The unbound matrices are washed away and a HRP-conjugated monoclonal antibody which specifically recognizes the protein of *Cryptosporidium parvum* is added for further immunoreactions. After an incubation period, an immunocomplex of "Anti-Cryptosporidium Antibody – *Cryptosporidium parvum* Antigen – HRP-conjugated Anti-Cryptosporidium Tracer Antibody" is formed if *Cryptosporidium parvum* antigen is 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 C. parvum proteins captured on the wall of each microtiter well is directly proportional to the amount of *Cryptosporidium parvum* 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-Cryptosporidium Antibody Coated Microplate (30456)

Coated with highly purified Anti-Cryptosporidium antibody.

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

2. Anti-Cryptosporidium Tracer Antibody (30457)

Concentrated horseradish peroxidase (HRP)-conjugated monoclonal Cryptosporidium antibody in a stabilized protein matrix.

Qty: 1 x 0.6 mL
Storage: 2 – 8°C
Preparation: Must be diluted with Tracer Antibody Dilutent (30458) prior to use.

3. Tracer Antibody Diluent (30458)

For antibody dilution according to the assay procedures.

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

4. ELISA Wash Concentrate (10010)

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.

5. ELISA HRP Substrate (10020)

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.

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

6. ELISA Stop Solution (10030)

0.5 M sulfuric acid.

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

7. Cryptosporidium Antigen Controls (30470, 30471)

Controls are in a liquid bovine serum albumin-based matrix with a non-azide preservative. The positive control is a dilution of highly purified inactivated Cryptosporidium parvum oocysts.

Qty: 2 x Vials
Storage: 2 – 8°C, <-20°C 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 preservative.

Qty: 1 x 30 mL
Storage: 2 – 8°C
Preparation: 20X Concentrate. The contents must be diluted with 570 mL distilled water and mixed well before use.

SAFETY PRECAUTIONS

The reagents are for in vitro diagnostic 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 were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. 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. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes.
5. Disposable plastic 1000 mL bottle with cap.
6. Aluminum foil.
7. Deionized or distilled water.
8. Plastic microtiter well cover or polyethylene film.
9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

SPECIMEN COLLECTION & STORAGE

Fresh fecal sample should be collected by using a plastic sampling device. The collected fecal sample must be transported, kept at 2-8°C and tested within 2 days. A non-preserved sample must be stored below -20°C for a longer storage period.

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 (10010) must be diluted to working solution prior to use. Please see REAGENTS section for details.

2. Sample Preparation

1. Label a test tube (12x75 mm) or a 1.5 ml plastic vial.
2. Add **1 mL** of 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** diluted patient sample diluent (30189) and mix well on a vortex mixer.
5. Centrifuge the diluted fecal sample at **3000** rpm (1500 g) for **10 – 15** minutes. The supernatant can be directly used in the assay. As an alternative to centrifuging, let the diluted samples sit and sediment for 15 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.

3. Assay Procedure

1. Place a sufficient number of microwell strips (30456) in a holder to run controls (30470, 30471) and diluted samples in duplicate.
2. Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Negative Control	SAMPLE 3	SAMPLE 7
B	Negative Control	SAMPLE 3	SAMPLE 7
C	Positive Control	SAMPLE 4	SAMPLE 8
D	Positive Control	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 controls (30470, 30471) and diluted samples into the designated microwells.
4. Cover the plate with one plate sealer and aluminum foil. Incubate 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.
6. Prepare the antibody working solution by 1:21 fold dilution of the tracer antibody (30457) with the diluent (30458). For each strip, it is required to mix 1 mL of the tracer antibody diluent with 50 µL of the tracer antibody in a clean test tube. *Note: This antibody working solution should be freshly prepared.*
7. Add **100 µL** of *antibody working solution* to each well. Mix by gently tapping the plate.
8. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **40 minutes.**
9. 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, then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
10. Add **100 µL** of ELISA HRP Substrate (10020) into each of the wells. Mix by gently tapping the plate.
11. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **15 minutes.**
12. Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution (10030) into each of the wells. Mix by gently tapping the plate.

13. Read the absorbance at **450 nm** within **10 minutes** with 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 zipper 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. All reagents should be mixed gently and thoroughly prior use. Avoid foaming.

INTERPRETION OF RESULTS

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 + 0.10)

   Negative Cut-Off = 0.9 x (mean extinction of negative control + 0.10)

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
   - Positive control must show an average OD reading greater than 0.500.
   - Negative control should show an average OD reading less than 0.200.

LIMITATIONS OF THE PROCEDURE

1. The results obtained with this Fecal Cryptosporidium parvum antigen test kit serve only as a useful aid to diagnosis. However, the test results should not be interpreted as diagnostic in themselves.
2. Bacterial or fungal contamination of stool specimens or reagents, or cross-contamination between reagents may cause erroneous results.
3. Water deionized with polyester resins may deactivate the horseradish peroxidase enzyme.

QUALITY CONTROL

To assure the validity of the results each assay must include both negative and positive controls. For a valid test, the positive control must have an absorbance of at least 0.5 OD units and the negative control must be less than 0.2 OD units. We also recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

EXAMPLE DATA

A typical absorbance data and the resulting negative control and positive control are represented.

Note: This absorbance must not be used in lieu of control values run with each assay.

	OD 450 nm	Average OD 450 nm
Negative Control	0.088 0.088	0.088
Positive Control	1.803 1.714	1.772

PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of this fecal Cryptosporidium parvum antigen ELISA is about 5 ng/ml of Cryptosporidium parvum antigen as determined by testing a series of dilutions of a highly purified sample of Cryptosporidium parvum antigen with assay buffer and the OD reading is above the positive cut-off.

Specificity

The assay does not cross react to following organisms: Giardia, *Rotavirus, and Adenovirus*.

Reproducibility and Precision

The reproducibility of this assay is validated by measuring four samples (two negative and two positive) both in a single assay of 12-replicate determinations and in 6 different assays run on different dates. The results showed a consistent test results interpretation for all the samples.

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

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.

This product is developed and manufactured by

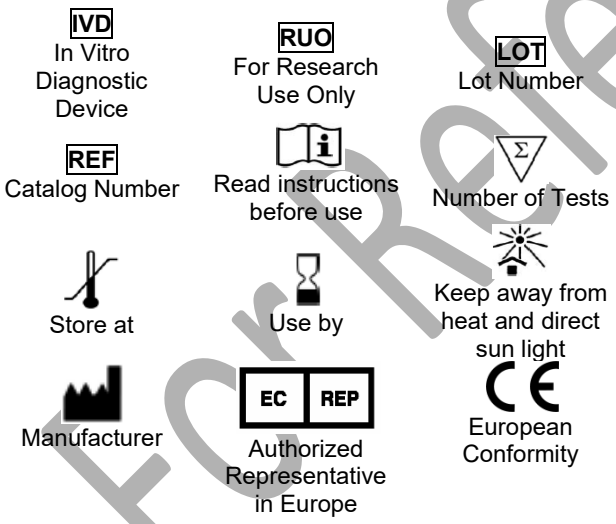
Epitope Diagnostics, Inc.
7110 Carroll Road
San Diego, CA 92121, US

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

EC	REP

MDSS GmbH
Schiffgraben 41,
30175 Hannover, Germany

GLOSSARY OF SYMBOLS (EN 980/ISO 15223)



SHORT ASSAY PROCEDURE

1. Add **100 µL** of the controls and diluted samples into the designated microwells.
2. Mix, cover, and incubate at **room temperature (20-25 ºC)** for **60 minutes**.
3. Wash each well five times.
4. Add **100 µL** of the working tracer antibody to each well.
5. Cover and incubate at **room temperature (20-25 ºC)** for **40 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 ºC)** for **15 minutes**.
9. Add **100 µL** of the stop solution to each well.
10. Read the absorbance at **450 nm**.