Datasheet for ABIN1305153

Cryptosporidium Parvum ELISA Kit

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Overview

Quantity:	96 tests
Target:	Cryptosporidium Parvum
Reactivity:	Cryptosporidium parvum
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	This microplate-based ELISA (enzyme linked immunosorbent assay)kit is intended for the qualitative detection of Cryptosporidiumparvum antigen in feces. The assay is a useful tool in the diagnosisof active Cryptosporidium parvum infection in acute or chronicdiarrhea.
Brand:	ED™
Sample Type:	Fecal
Analytical Method:	Qualitative
Detection Method:	Colorimetric
Specificity:	The assay does not cross react to following organisms: Giardia, Rotavirus, and Adenovirus.
Characteristics:	Gastrointestinal Disease
Components:	1. Anti-Cryptosporidium Antibody Coated Microplate. One vial contains Cryptosporidium negative control (30471) andanother vial contains inactivated Cryptosporidium positivecontrol (30470). Both controls are in a liquid bovine serumalbumin-based matrix with a non-azide preservative. Thepositive control is a dilution of highly purified Cryptosporidiumparvum oocysts. Refer to vials for exact concentrationrange for each control. After the first use, the

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	controls shouldbe stored at -20 °C or below for long-term storage.
Material not included:	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.

Target Details

Target:	Cryptosporidium Parvum
Abstract:	Cryptosporidium Parvum Products
Target Type:	Species
Background:	Cryptosporidiosis is one of the main causes of persistent diarrhea inthe developed world. It is caused by the presence ofCryptosporidium parvum oocysts in the gastro-intestinal tract. Thisparasite is known to be highly pathogenic and its infectious stage istransmitted by faecal-oral contract. It is also an opportunisticpathogen found in immunocompromised patients.The symptoms of cryptosporidiosis are watery diarrhea, stomachcramps, weight loss, nausea, and fever1. In industrialized countries,2-2.5 % of diarrhreal hospitalized patients shed C. parvum oocysts.Ten percent of AIDS patients have chronic cryptosporidiosis and thisfigure can be as high as 40 % in certain developing countries.C. parvum is diagnosed by either Ziehl-Neelsen stain orimmunofluorescence in smears of unconcentrated specimens.

Application Details

Sample Volume:	0.1 mL
Assay Time:	4 h
Plate:	Pre-coated
Protocol:	This sandwich ELISA is designed, developed and produced for thequalitative measurement of Cryptosporidium parvum antigen in stoolspecimen. The assay utilizes the microplate-based

	enzymeimmunoassay technique by coating highly purified antibody onto thewall of microtiter well.Assay controls and fecal specimen are added to microtiter wells ofmicroplate that was coated with a highly purified polyclonal anti-Cryptosporidium parvum antibody on its wall. The Cryptosporidiumparvum antigen will be bound to the antibody coated plate after anincubation period. The unbound matrices are washed away and aHRP-conjugated monoclonal antibody which specifically recognizesthe protein of Cryptosporidium parvum is added for furtherimmunoreactions. After an incubation period, an immunocomplex ofAnti-Cryptosporidium Antibody Cryptosporidium parvum Antigen HRP-conjugated Anti-Cryptosporidium Tracer Antibody is formed ifCryptosporidium parvum antigen is present in the test sample. Theunbound tracer antibody and other protein or buffer matrix areremoved in the subsequent washing step. HRP-conjugated tracerantibody bound to the well is then incubated with a substrate solutionin a timed reaction and then measured in a spectrophotometricmicroplate reader. The enzymatic activity of the tracer antibodybound to C. parvum proteins captured on the wall of each microtiterwell is directly proportional to the amount of Cryptosporidium parvumantigen level in each test specimen.
Reagent Preparation:	Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
Sample Collection:	Fresh fecal sample should be collected by using a plastic sampling device, for example, Epitope Diagnostics Fecal Sample Collection Device. It is required to collect a minimum of 0.1 mL liquid stool sample or 0.1 g solid sample. 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.
Sample Preparation:	(1) Label a test tube (12x75 mm) or a 1.5 mLplastic vial. (2) Add 1 mL of assay buffer 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 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.
Assay Procedure:	(1) Place a sufficient number of Anti-Cryptosporidium antibody coated microwell strips in a frame to run Cryptosporidium controls and unknown samples in duplicate.

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	(2) Test Configuration (3) Add 100 µL of controls (Cat. 30470-30471) and diluted patient stool samples into each designated microwell. (4) Cover the plate with a plate sealer and also with aluminum foil to avoid exposure to light. (5) Incubate plate at room temperature for 1 hour. (6) Prepare working Anti-Cryptosporidium tracer antibody working solution by 1:21 fold dilution of the Anti-Cryptosporidium Tracer Antibody with the Tracer Antibody Diluent. For each strip, it is required to mix 1 mL of Tracer Antibody Diluent with 50 µL of Tracer Antibody in a clean test tube. (7) Remove the plate sealer. Decant the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of diluted wash buffer into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used. (8) Add 100 µL of above diluted tracer antibody working solution to each of the wells. (9) Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light. (10) Incubate plate at room temperature for 40 minutes. (11) Remove the plate sealer. Decant the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of diluted wash buffer into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used. (12) Add 100 µL of ELISA HRP Substrate into each of the wells. (13) Cover the plate with aluminum foil to avoid exposure to light. (14) Incubate plate at room temperature for 15 minutes (15) Remove the aluminum foil. Add 100 µL of ELISA Stop Solution into each of the wells. Mix gently. (16) Read the absorbance at 450 nm within 10 minutes in a microplate reader.
Calculation 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 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.
Restrictions:	For Research Use only

Handling

Precaution of Use:	The reagents must be used in a laboratory and are for professional use only. Source material for reagents containing 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 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.
Storage:	4 °C