Datasheet for ABIN1305156

S100A8/A9 Complex (Calprotectin) ELISA Kit

2 Images

Go to Product page

Overview

Quantity:	96 tests
Target:	S100A8/A9 Complex (Calprotectin) (S100A8/A9)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0 µg/mL - 2000 µg/mL
Minimum Detection Limit:	2.5 ng/mL
Application:	ELISA

Product Details

Purpose:	This test kit is intended for use in the quantitative determination of human calprotectin (neutrophil cytoplasmic protein S100A8/A9) levels in stool samples. It is for in-vitro diagnostic use. The test is useful for detecting inflammatory bowel disease (IBD) such as ulcerative colitis and Crohn's disease.
Brand:	ED™
Sample Type:	Fecal
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	1. Calprotectin Antibody Coated Microplate One bottle containing 30 mL of 20-fold concentrate. Before use the contents must be diluted with 570 mL of demineralized water and mixed well. Upon dilution, this yields a ready-to-use Extraction Buffer for fecal sample extraction and dilution. The diluted Extraction Buffer may be

Product Details

	stored at room temperature and is stable until the expiration date on the kit box.
Material not included:	1. Fecal sample collection tube 2. Precision single channel pipettes capable of delivering 50 µL, 100 µL, 500 µL, etc. 3. Disposable pipette tips suitable for above volume dispensing. 4. Disposable plastic 100 mL and 1000 mL bottle with caps. 5. Aluminum foil. 6. Deionized or distilled water. 7. Plastic microtiter well cover or polyethylene film. 8. ELISA multichannel wash bottle or automatic (semi-automatic) washing system. 9. Spectrophotometric microplate reader capable of reading absorbance at 450 nm and 650 or 630

Target Details

Target:	S100A8/A9 Complex (Calprotectin) (S100A8/A9)
Alternative Name:	Calprotectin (S100A8/A9 Products)
Background:	Quantitative determination of fecal calprotectin is an indication of the severity of bowel inflammation. Also, higher levels of calprotectin in the stool are associated with an increased risk of relapse in patients with inflammatory bowel disease (IBD).1 Low stool calprotectin levels correlate well with a low risk for intestinal allograft rejection. This assay uses specific monoclonal antibodies to ensure only calprotectin is detected.
Pathways:	S100 Proteins

Application Details

Assay Time:	4 h
Plate:	Pre-coated
Protocol:	This ELISA is designed, developed and produced for the quantitative measurement of human calprotectin in stool samples. The assay utilizes the two-site sandwich technique with two selected antibodies that bind to different epitopes of human calprotectin. Assay standards, controls and patient samples are added directly to wells of a microtiter plate that is coated with antibody to calprotectin. After a short incubation period, the plate is washed and horseradish peroxidase (HRP)-conjugated human calprotectin specific monoclonal antibody is added to each well. After the second incubation period, a sandwich of solid-phase antibody human calprotectin HRP-conjugated monoclonal antibody is formed. The unbound monoclonal

	antibodies and buffer matrix are removed in the subsequent washing step. For the detection of this immunocomplex, 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 immunocomplex bound to the wall of each microtiter well is directly proportional to the amount of human calprotectin in the test sample. A standard curve is generated by plotting the absorbance versus the respective human calprotectin concentration for each standard on a point-to-point or 4-parameter curve fitting. The concentration of fecal human calprotectin in test samples is determined directly from this standard curve.
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 must be diluted to working solution prior to use. Please see REAGENTS section for details. (3) Reconstitute all assay standard level 1 to level 7 and controls by adding 0.5 mL of deminerialized water to each vial. Allow the standards and controls to sit undisturbed for 5 minutes, and then mix well by inversions or gentle vortexing. One must make sure that all solid is dissolved completely prior to use. These reconstituted standards and controls may be stored at 2 ? 8 C for up to 3 days or at ?10 C or below for long-term storage. Do not exceed 3 freeze-thaw cycles. (4) Test Configuration (5) Place a sufficient number of calprotectin-coated microwell strips in a holder to run human calprotectin standards, controls and unknown samples in duplicate. (6) Prepare Tracer Antibody working solution by 1:21 fold dilution of the Calprotectin Tracer Antibody by adding the tracer antibody into the Tracer Antibody Diluent . Following is a table that outlines the relationship of strips used and antibody mixture prepared.
Sample Collection:	1. Only one fecal sample is required. Fresh fecal sample must be collected by using Epitope Diagnostics Fecal Sample Collection Tube. This tube is specially designed for easy collection of a substantially small amount of fecal sample into the tube pre-filled with sample extraction buffer. The collected fecal sample may be transported at ambient temperature, stored at 2-8 C and tested within 3 days. Fecal sample may be stored below -20 C for a longer storage period. Avoid more than three freeze - thaw cycles for each specimen. The validation data of this test were generated by using Fecal Sample Collection Tube! To order this tube, please order Fecal Calprotectin/NGAL Sample Collection kit. Each kit contains 50 tubes filled with extraction buffer. A different calprotectin test result may be obtained by using a different type of fecal sample collection tube. 2. It is an alternative to collect fecal sample with a commercial stool sample collection device.

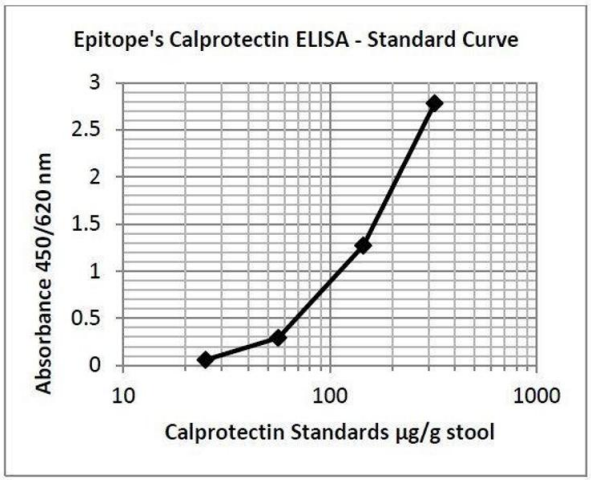
	The collected sample can be stored at 2-8 °C for up to 6 days. The collected sample should be diluted in two steps with 1:40 and 1:9 before measurement. Following is a detailed sample extraction process. (a) Label and tare an empty polypropylene tube together with an inoculation loop. (b) Weigh 50 ? 100 mg of stool using the inoculation loop by placing it into the pre-tarred tube. (c) Record the net amount of sample and break the inoculation loop, leave the lower part of the loop in the tube. (d) Add Extraction Buffer (39 parts of the stool volume, 1 g stool = 1 ml) into the tube: (e) Vortex to dissolve stool sample. Let the sample set at room temperature vertically for 30 min for sedimentation or centrifuge the sample at 3000 x g for 5 minutes. (f) Transfer 0.15 mL clear supernatant (no particles) to a clean tube with 1.2 mLExtraction Buffer. Mix the sample by gently vortexing. This extracted sample is ready to be measured for fecal Calprotectin.
Sample Preparation:	If the Epitope Diagnostics Fecal Sample Collection Tube is used, there is no sample preparation required.
Assay Procedure:	(1) Add 50 µL of Assay Buffer into the designated microwells. Gently tap the plate to coat the wells evenly. (2) Add 50 µL of Standards, Controls and extracted patient samples into the designated microwells. (3) Seal the plate wells securely, cover with foil or other material to protect from light, and rotate on an ELISA plate shaker (small orbit radius) for 1 hr. 5 minutes at 400 to 450 rpm. (4) Just prior to the end of the incubation time, dilute the proper amount of Tracer Antibody for the assay. (5) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used. (6) Add 100 µL of diluted Tracer Antibody to each well. (7) Seal the plate wells securely, cover with foil or other material to protect from light, and rotate on an ELISA plate shaker (small orbit radius) for 45 minutes 5 minutes at 400 to 450 rpm. (8) Wash each well 5 times by dispensing 350 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 into each of the wells. (10) Cover the plate with aluminum foil to or other material to avoid exposure to light. Incubate plate static, at room temperature, for 12 minutes (Optional 8 - 15 minutes). (11) Remove the aluminum foil. Read the absorbance at 620 nm (optional wavelengths from 595 nm to 650 nm depending on available filters) immediately.

Application Details

	(12) Immediately add 100 µL of ELISA Stop Solution into each of the wells. Mix gently. (13) Read the absorbance at 450 nm with reference filter at 620 nm or 650 nm.
Calculation of Results:	It is recommended to use a point-to-point or 4-parameter standard curve fitting. 1. Calculate the average absorbance for each pair of duplicate test results. 2. Subtract the average absorbance of the level 1 standard (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance. 3. The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard 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. The fecal human calprotectin concentrations for the controls and the patient samples are read directly from the standard curve using their respective corrected absorbance. The use of the two absorbance wavelength at A 620 nm and A450/620 nm allows for two ways to calculate sample results. It is recommended to get sample results by using the primary standard curve at A 450/620 nm for samples with value below standard level 5. For samples with calprotectin value above standard level 5, it is recommended to use the secondary standard curve at A 620 nm.
Assay Precision:	The intra-assay precision was validated by measuring three sample extracts in a single assay with 12 replicate determinations. The inter-assay precision was validated by measuring two samples in duplicate in 4 individual assays.
Restrictions:	For Research Use only

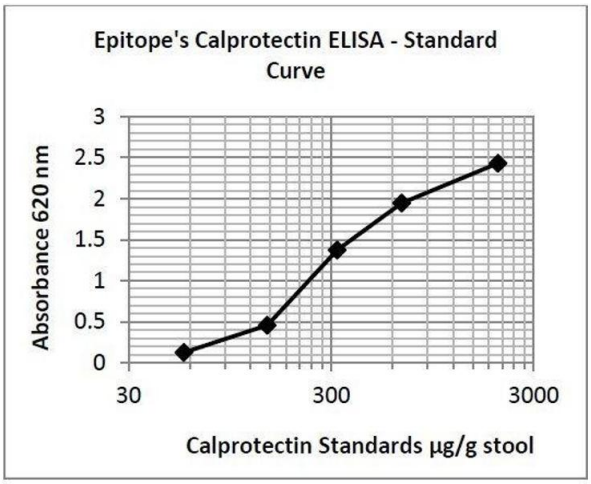
Handling

Precaution of Use:	The reagents must be used in a professional laboratory. 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 hydrochloric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Hydrochloric 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



ELISA

Image 1.



ELISA

Image 2.