Datasheet for ABIN1305158

Myeloperoxidase ELISA Kit

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Overview

Quantity:	96 tests
Target:	Myeloperoxidase (MPO)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.15-256 µg/g
Minimum Detection Limit:	0.15 µg/g
Application:	ELISA

Product Details

Purpose:	This test kit is intended for use in the quantitative determination of human myeloperoxidase (MPO) levels in stool and urine samples. The test is useful for detecting elevated levels of myeloperoxidase in stool samples, which may serve as a sensitive predictor for inflammatory activities in the gastrointestinal tract.
Brand:	ED™
Sample Type:	Fecal, Urine
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	1. Myeloperoxidase Antibody Coated Microplate Two bottles containing 30 mL of fecal sample extraction buffer. This is a ready-to-use Extraction Buffer for fecal sample extraction and standard dilution. The Fecal Sample Extraction Buffer may be stored at room temperature and is stable until the expiration date on

Product Details

	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 405/650 nm.

Target Details

Target:	Myeloperoxidase (MPO)
Alternative Name:	Myeloperoxidase (MPO Products)
Background:	Myeloperoxidase (MPO) is a specific polymorphonuclear enzyme that is most abundantly expressed in neutrophil granulocytes. It functions in the oxygen-dependent killing of microorganisms, and is released from primary granules of neutrophils during acute inflammation. MPO is the product of a single gene, which is about 11 kb in size, composed of 11 introns and 12 exons, and located in the long arm of chromosome 17 in segment q12-24. The mature 150 kDa MPO protein is a dimer consisting of two 15 kDa light chains and two heavy chains of variable degrees of glycosylation. MPO activity was found to be linearly related to the number of neutrophil cells. Since neutrophils play a predominant role in inflammatory and immune reactions in inflammatory bowel disease (IBD), and MPO has been observed both in the intestinal mucosa and the gut lavage, the determination of MPO in stool sample provides one of the most sensitive and promising biomarkers in predicting disease severity. This assay utilizes a specific monoclonal antibody to capture MPO in test sample to ensure that only myeloperoxidase is detected.
Pathways:	Chromatin Binding

Application Details

Assay Time:	4 h
Plate:	Pre-coated
Protocol:	This ELISA kit is designed, developed and produced for the quantitative measurement of

	human myeloperoxidase in stool samples. The assay utilizes the two-site sandwich technique with selected antibodies that bind to different epitopes of myeloperoxidase.Assay standards, controls and extracted patient samples are added directly to wells of a microtiter plate that is coated with antibody to myeloperoxidase. After an incubation period, the plate is washed and horseradish peroxidase (HRP)-conjugated human myeloperoxidase antibody is added to each well. After the second incubation period, a sandwich of solid-phase monoclonal antibody human myeloperoxidase HRP-conjugated antibody is formed. The unbound 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 the absorbances are 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 myeloperoxidase in the test sample. A standard curve is generated by plotting the absorbance versus the respective human myeloperoxidase concentration for each standard on a point-to-point or 4-parameter curve fitting. The concentration of human myeloperoxidase 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 use. Please see REAGENTS section for details. (3) Reconstitute assay standard by adding 2.0 mL of deminerialized water to standard vial. Separately, reconstitute controls by adding 1.0 mL of deminerialized water to control vials. Allow the standard and controls to sit undisturbed for 5 minutes, and then mix well by inversions or gentle vortexing. Make sure that all solid is dissolved completely prior to use. These reconstituted standard 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) Dilute the reconstituted standard concentrate 1:4 using the fecal sample extraction buffer to obtain a level six standard by mixing the concentrated MPO standard with the fecal sample extraction buffer. For example: mix 300 L of concentrated MPO standard with 900 L of the extraction buffer. Continue diluting standards down to level two as it is shown below. Level one standard is the fecal sample extraction buffer. (5) Test Configuration (6) Place a sufficient number of myeloperoxidase antibody coated microwell strips in a holder to run human myeloperoxidase standards, controls and unknown samples in duplicate. (7) Prepare Tracer Antibody working solution by 1:21 fold dilution of the Myeloperoxidase 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. Fecal sample collection 1.1 Only one fecal sample is required. Fresh fecal sample must be collected by using Epitope Diagnostics Fecal MPO 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 Myeloperoxidase Sample Collection kit. Each kit contains 50 tubes that are pre-filled with fecal sample extraction buffer. A different myeloperoxidase test result may be obtained by using a different type of fecal sample collection tube and extraction buffer. 1.2. 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-tared 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 (49 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 mLclear 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 myeloperoxidase. 2. Urine sample collection A standard urine collection container must be used to collect a fresh and random urine sample.
Assay Procedure:	(1) Add 100 µL of Standards, Controls and extracted patient samples into the designated microwells. (2) 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 1.5 hr. 5 minutes at 400 to 450 rpm. (3) Just prior to the end of the incubation time, dilute the proper amount of Tracer Antibody for the assay. (4) 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. (5) Add 100 µL of above Tracer Antibody to each well.

Application Details

	(6) 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. (7) 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. (8) Add 100 µL of ELISA HRP Substrate into each of the wells. (9) Cover the plate with aluminum foil to or other material to avoid exposure to light. Incubate plate static, at room temperature, for 20 minutes. (10) Immediately add 100 µL of ELISA Stop Solution into each of the wells. Mix gently. (11) Read the absorbance at 405 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 g/g) 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 myeloperoxidase concentrations for the controls and the patient samples are read directly from the standard curve using their respective corrected absorbance.
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 three samples in duplicate in 8 individual assays.
Restrictions:	For Research Use only

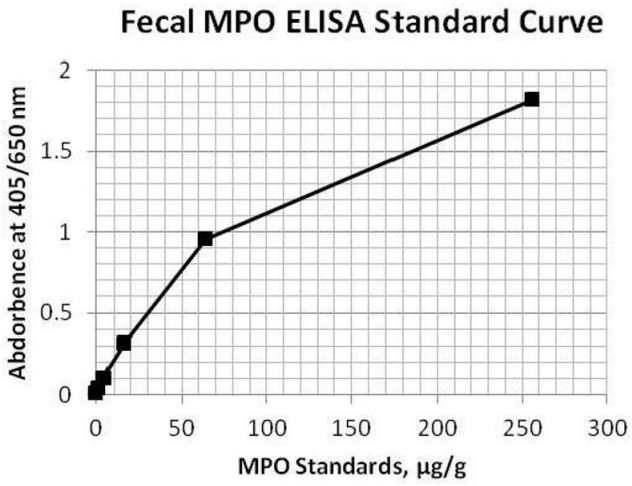
Handling

Precaution of Use:	The reagents must be used in a professional laboratory. 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

Handling

	fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.
Storage:	4 °C

Images



ELISA

Image 1.