Datasheet for ABIN2014342

# Lipocalin 2 ELISA Kit

1 Image

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## Overview

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| Quantity: | 96 tests |
| Target: | Lipocalin 2 (LCN2) |
| Reactivity: | Human |
| Method Type: | Sandwich ELISA |
| Detection Range: | 0.04-97.2 µg/g |
| Minimum Detection Limit: | 0.04 µg/g |
| Application: | ELISA |

## Product Details

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| Purpose: | This test kit is intended for use in the quantitative determination of human neutrophil gelatinase-associated lipocalin (Lipocalin-2 or NGAL) in feces. NGAL is extremely stable in feces.Indications for use: 1. Patient may have an abnormally high level of NGAL in feces with active inflammatory bowel diseases (IBD), such as crohn's disease, colitis ulcer, etc.2. Patient may have an abnormally high level of NGAL in urine with acute kidney failure. |
| Brand: | EDI™ |
| Sample Type: | Fecal |
| Analytical Method: | Quantitative |
| Detection Method: | Colorimetric |
| Characteristics: | This ELISA kit is designed, developed and produced for the quantitative measurement of human NGAL in stool samples. The assay utilizes the sandwich technique with selected antibodies that bind to various epitopes of NGAL. Assay standards, controls and patient |

## Product Details

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| | samples are added directly to wells of a microtiter plate that is coated with antibody to human NGAL and incubated at room temperature for one hour. The plate is then washed and horseradish peroxidase (HRP) conjugated anti NGAL is added to each well. After an additional incubation period, a ?sandwich? of solid-phase polyclonal antibody - human NGAL 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, which is terminated with an acidic reagent (i.e. ELISA stop solution). The absorbance is 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 NGAL in the test sample. A standard curve is generated by plotting the absorbance versus the respective human NGAL concentration for each standard on a point-to-point or 4-parameter curve fitting. The concentration of human NGAL in test samples is determined directly from this standard curve. |
| Components: | 1. Anti-NGAL Antibody Coated Microplate<br>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 stored at 2-8 ?C and is stable for 2 months. |
| Material not included: | 1. Fecal sample collection tube<br>2. Precision single channel pipettes capable of delivering 100 µL.<br>3. Disposable pipette tips suitable for above volume dispensing.<br>4. Aluminum foil.<br>5. Deionized or distilled water.<br>6. Plastic microtiter well cover or polyethylene film.<br>7. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.<br>8. Spectrophotometric microplate reader capable of reading absorbance at 450/650 or 450/620 nm. |

## Target Details

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| Target: | Lipocalin 2 (LCN2) |
| Alternative Name: | Neutrophil Gelatinase-Associated Lipocalin (LCN2 Products) |
| Pathways: | Cellular Response to Molecule of Bacterial Origin, Transition Metal Ion Homeostasis |

## Application Details

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| Assay Time: | 4 h |
| Plate: | Pre-coated |
| Protocol: | This ELISA kit is designed, developed and produced for the quantitative measurement of human NGAL in stool samples. The assay utilizes the sandwich technique with selected antibodies that bind to various epitopes of NGAL.Assay standards, controls and patient samples are added directly to wells of a microtiter plate that is coated with antibody to human NGAL and incubated at room temperature for one hour. The plate is then washed and horseradish peroxidase (HRP) conjugated anti NGAL is added to each well. After an additional incubation period, a sandwich of solid-phase polyclonal antibody - human NGAL 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, which is terminated with an acidic reagent (i.e. ELISA stop solution). The absorbance is 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 NGAL in the test sample. A standard curve is generated by plotting the absorbance versus the respective human NGAL concentration for each standard on a point-to-point or 4-parameter curve fitting. The concentration of human NGAL 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.<br>(2) ELISA Wash Concentrate must be diluted to working solution prior to use. Please see REAGENTS section for details.<br>(3) Reconstitute assay standards and controls by adding 1.0 mL of deminerialized water to each standard and control bottle. 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 standards and controls may be stored at 2- 8 C for up to 3 days or below ?20 C for long-term storage. Do not exceed 3 freeze-thaw cycles<br>(4) Place a sufficient number of Anti-NGAL antibody-coated microwell strips in a holder to run human NGAL standards, controls and unknown samples in duplicates.<br>(5) Prepare Tracer Antibody working solution by 1:21 fold dilution of the NGAL 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 |

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| | 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 room temperature or 2-8 C for 14 days. This fecal sample may be stored below -20 C for a 1 year and is stable minimum with three freeze - thaw cycles. 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 fecal NGAL test result may be obtained by using a different type of fecal sample collection tube.<br>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 1:90 before measurement. Following is a detailed sample extraction process. (a) Label and tare an empty polypropylene tube together with a 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 diluted 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 0.015 mL clear supernatant (no particles) to a clean tube with 1.35 mLExtraction Buffer. Mix the sample by gently vortexing. This extracted sample is ready to be measured for fecal NGAL. |
| Sample Preparation: | 1. Patient samples collected with Fecal Sample Collection Tube .All patient samples should be diluted 1:10 with 1x Calprotectin/NGAL Sample Extraction Buffer. For example, mixing 100 µL sample with 900 µL buffer in a clean test tube. This diluted sample can be used directly in the assay procedure.<br>2. Patient samples collected and extracted according to the specimen collection section<br>2.These samples don't require any further dilution and can be used directly in the assay procedure. |
| Assay Procedure: | (1) Add 100 µL of Standards, Controls and diluted patient samples (diluted beforehand with NGAL Sample Dilution Buffer, Cat. 30757) into the designated microwells.<br>(2) Seal the plate wells securely, cover with foil or other material to protect from light. Incubate the plate static, at room temperature for 1 hr. 5 minutes.<br>(3) Just prior to the end of the incubation time, dilute the proper amount of Tracer Antibody for the assay.<br>(4) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and |

## Application Details

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| | then completely aspirating the contents. Alternatively, an automated microplate washer can be used.<br>(5) Add 100 µL of the above Tracer Antibody to each well.<br>(6) Seal the plate wells securely, cover with foil or other material to protect from light. Incubate the plate static, at room temperature for 30 minutes 5 minutes.<br>(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.<br>(8) Add 100 µL of ELISA HRP Substrate into each of the wells.<br>(9) Cover the plate with aluminum foil or other material to avoid exposure to light. Incubate the plate static, at room temperature for 20 minutes.<br>(10) Immediately add 100 µL of ELISA Stop Solution into each of the wells. Mix gently.<br>(11) 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.<br>1. Calculate the average absorbance for each pair of duplicate test results.<br>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.<br>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 human NGAL 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 control fecal samples with 16 replicate determinations. The inter-assay precision was validated by measuring two control levels in duplicate in 14 individual assays. |
| Restrictions: | For Research Use only |

## Handling

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| Precaution of Use: | The reagents must be used in 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 sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. |

## Handling

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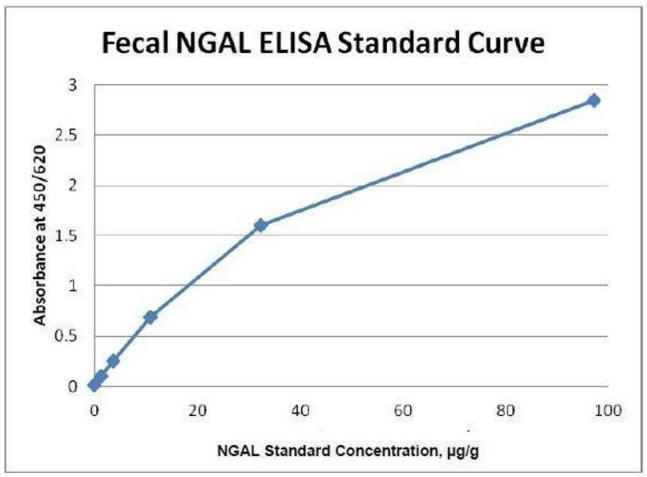
| | TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices. |
| Storage: | 4 °C |

## Images




**ELISA**

**Image 1.**