Calprotectin ELISA

Catalog Number:
CAL35-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 2.0 (08.16.17)
INTENDED USE
The Eagle Biosciences Calprotectin ELISA Assay kit is intended for use in the quantitative
determination of human calprotectin (neutrophil cytoplasmic protein S100A8/A9) levels in stool
samples via Enzyme-linked immunosorbent assay. The test is useful for detecting inflammatory
bowel disease (IBD) such as ulcerative colitis and Crohn’s disease. The Calprotectin ELISA Assay
kit is for research use only and should not be used in diagnostic procedures.

INTRODUCTION
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.

ASSAY PRINCIPLE
This Calprotectin ELISA Assay Kit is designed, developed and produced for the quantitative
measurement of human calprotectin in stool samples. The Calprotectin ELISA Assay Kit 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 of the Calprotectin ELISA Assay.

REAGENT Preparation and Storage
This Calprotectin ELISA Assay 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.

Prior to use allow all reagents to come to room temperature. Regents from different kit lot
numbers should not be combined or interchanged.

1. Calprotectin Antibody Coated Microplate
   One microplate with twelve by eight strips (96 wells total) coated with calprotectin antibody.
The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be
stored at 2 – 8 °C and is stable until the expiration date on the Calprotectin ELISA Assay Kit
box.

2. Calprotectin Tracer Antibody
   One vial containing 0.6 mL HRP labeled anti-human calprotectin antibody in a stabilized
protein matrix. This reagent must be diluted with Tracer Antibody Diluent before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the Calprotectin ELISA Assay Kit box.

3. **ELISA Wash Concentrate**
   One bottle contains 30 mL of 30-fold concentrate. Before use the contents must be diluted with 870 mL of demineralized water and mixed well. Upon dilution, this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide, non-mercury preservative. The diluted wash solution may be stored at room temperature and is stable until the expiration date on the Calprotectin ELISA Assay Kit box.

4. **ELISA HRP Substrate**
   One bottle contains 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the Calprotectin ELISA Assay Kit box.

5. **ELISA Stop Solution**
   One bottle contains 12 mL of 2N Hydrochloric Acid (HCl). This reagent may be stored at 2 – 8°C or room temperature and is stable until the expiration date on the Calprotectin ELISA Assay Kit box.

6. **Calprotectin Standards**
   Seven vials containing human calprotectin in a lyophilized bovine serum based matrix with a non-azide, non-mercury preservative. Refer to vials for exact concentration for each standard. These reagents should be stored at 2 – 8°C and are stable until the expiration date on the Calprotectin ELISA Assay Kit box.

7. **Calprotectin Controls**
   Three vials containing human calprotectin in a lyophilized bovine serum based matrix with a non-azide, non-mercury preservative. Refer to vials for exact concentration range for each control. Both controls should be stored at 2 – 8°C and are stable until the expiration date on the Calprotectin ELISA Assay Kit box.

8. **Tracer Antibody Diluent**
   One vial containing 12 mL ready to use buffer. It should be used only for tracer antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the Calprotectin ELISA Assay Kit box.

9. **Assay Buffer**
   One bottle containing 12 mL ready to use buffer. It should be used according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the Calprotectin ELISA Assay Kit box.

10. **Extraction Buffer Concentrate**
    One bottle containing 120 mL of 5-fold concentrate. Before use the contents must be diluted with 480 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 room temperature and is stable until the expiration date on the Calprotectin ELISA Assay Kit box.
SAFETY PRECAUTIONS
The reagents of the Calprotectin ELISA Assay Kit must be used in 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 potential 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.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Fecal sample collection tube (Catalog Number CAL35-C50)
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

SPECIMEN COLLECTION
1. Only one fecal sample is required. Fresh fecal sample must be collected by using Eagle Biosciences Fecal Sample Collection Tube (Cat. No. CAL35-C50). 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 times freeze - thaw cycle for each specimen.

The validation data of this Calprotectin ELISA were generated by using Fecal Sample Collection Tube. To order this tube, please order Fecal Calprotectin Sample Collection kit (Cat. No. CAL35-C50) and each kit contains 50 tubes. A different calprotectin test result may be obtained by using a different type of fecal sample collection tube.

2. Alternatively, 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 (39 parts of the stool volume, 1 g stool = 1 ml) into the tube:
### Fecal Sample Weight (mg) | Extraction Buffer Volume (ml)
---|---
50 - 54 | 2.0
55 - 59 | 2.2
60 - 64 | 2.4
65 - 69 | 2.6
70 - 74 | 2.8
75 - 79 | 3.0
80 - 84 | 3.2
85 - 89 | 3.4
90 - 94 | 3.6
95 - 99 | 3.8
100 - 104 | 4.0

(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 ml Extraction Buffer. Mix the sample by gently vortexing. This extracted sample is ready to be measured for fecal Calprotectin.

### ASSAY PROCEDURE

1. **Reagent Preparation**
   1. Prior to use allow all reagents of the Calprotectin ELISA Assay Kit to come to room temperature. Regents 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 all assay standard level 1 to level 7 and controls by adding 0.5 mL of demineralized 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

<table>
<thead>
<tr>
<th>ROW</th>
<th>STRIP 1</th>
<th>STRIP 2</th>
<th>STRIP 3</th>
<th>STRIP 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>STD 1</td>
<td>STD 5</td>
<td>C 2</td>
<td>SAMPLE 5</td>
</tr>
<tr>
<td>B</td>
<td>STD 1</td>
<td>STD 5</td>
<td>C 2</td>
<td>SAMPLE 6</td>
</tr>
<tr>
<td>C</td>
<td>STD 2</td>
<td>STD 6</td>
<td>C 3</td>
<td>SAMPLE 7</td>
</tr>
<tr>
<td>D</td>
<td>STD 2</td>
<td>STD 6</td>
<td>C 3</td>
<td>SAMPLE 8</td>
</tr>
<tr>
<td>E</td>
<td>STD 3</td>
<td>STD 7</td>
<td>SAMPLE 1</td>
<td>SAMPLE 9</td>
</tr>
<tr>
<td>F</td>
<td>STD 3</td>
<td>STD 7</td>
<td>SAMPLE 2</td>
<td>SAMPLE 10</td>
</tr>
<tr>
<td>G</td>
<td>STD 4</td>
<td>C 1</td>
<td>SAMPLE 3</td>
<td>SAMPLE 11</td>
</tr>
<tr>
<td>H</td>
<td>STD 4</td>
<td>C 1</td>
<td>SAMPLE 4</td>
<td>Etc.</td>
</tr>
</tbody>
</table>

(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.

<table>
<thead>
<tr>
<th>Strip no.</th>
<th>Tracer Antibody Diluent</th>
<th>Tracer Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 mL</td>
<td>50 µL</td>
</tr>
<tr>
<td>2</td>
<td>2 mL</td>
<td>100 µL</td>
</tr>
<tr>
<td>3</td>
<td>3 mL</td>
<td>150 µL</td>
</tr>
<tr>
<td>4</td>
<td>4 mL</td>
<td>200 µL</td>
</tr>
<tr>
<td>5</td>
<td>5 mL</td>
<td>250 µL</td>
</tr>
<tr>
<td>6</td>
<td>6 mL</td>
<td>300 µL</td>
</tr>
<tr>
<td>7</td>
<td>7 mL</td>
<td>350 µL</td>
</tr>
<tr>
<td>8</td>
<td>8 mL</td>
<td>400 µL</td>
</tr>
<tr>
<td>9</td>
<td>9 mL</td>
<td>450 µL</td>
</tr>
<tr>
<td>10</td>
<td>10 mL</td>
<td>500 µL</td>
</tr>
<tr>
<td>11</td>
<td>11 mL</td>
<td>550 µL</td>
</tr>
<tr>
<td>12</td>
<td>12 mL</td>
<td>600 µL</td>
</tr>
</tbody>
</table>

Note: this antibody working solution should be freshly prepared just before pipetting the tracer antibody to the washed wells.
2. **Patient Sample Preparation**  
If the Eagle Biosciences Fecal Sample Collection Tube is used, there is no sample preparation required.

3. **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 above 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.
   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.

**PROCEDURAL NOTES**
1. It is recommended that all standards, controls and unknown samples be assayed in duplicate with the Calprotectin ELISA Assay Kit. 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. An orbital mixer with a larger orbit radius (e.g. > 1 cm) may be used at speeds of 150 to 200 rpm.
7. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
8. All reagents of the Calprotectin ELISA Assay Kit should be mixed gently and thoroughly prior to use. Avoid foaming.
9. Adapting this assay to automated ELISA system such as DS-2, a procedural validation is necessary if there is any modification of the assay procedure.

INTERPRETATION 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 getting sample results by using the primary standard curve at A 450/620 nm for samples with value below standard level 5. While for samples Calprotectin value above standard level 5, it is recommend using the secondary standard curve at A 620 nm.

EXAMPLE DATA AND STANDARD CURVE (low)
A typical absorbance data and the resulting standard curve from this fecal human calprotectin ELISA are represented. This curve should not be used in lieu of standard curve run with each assay.

<table>
<thead>
<tr>
<th>Well I.D.</th>
<th>OD 450 nm Absorbance</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Readings</td>
<td>Average</td>
</tr>
<tr>
<td>Std-1: 0 µg/g (0 ng/mL)</td>
<td>0.026</td>
<td>0.027</td>
</tr>
<tr>
<td>Std-2: 25 µg/g (69.5 ng/mL)</td>
<td>0.061</td>
<td>0.059</td>
</tr>
<tr>
<td>Std-3: 56.2 µg/g (156 ng/mL)</td>
<td>0.305</td>
<td>0.292</td>
</tr>
<tr>
<td>Std-4: 145 µg/g (403 ng/mL)</td>
<td>1.388</td>
<td>1.272</td>
</tr>
<tr>
<td>Std-5: 321 µg/g (892 ng/mL)</td>
<td>2.760</td>
<td>2.781</td>
</tr>
<tr>
<td>Control 1</td>
<td>0.148</td>
<td>0.134</td>
</tr>
<tr>
<td>Control 2</td>
<td>2.601</td>
<td>2.607</td>
</tr>
</tbody>
</table>

36.1 µg/g (100 ng/ml)
291.4 µg/g (810 ng/ml)
**EXAMPLE DATA AND STANDARD CURVE** (high)

A typical absorbance data and the resulting standard curve from this fecal human calprotectin ELISA are represented. **This curve should not be used in lieu of standard curve run with each assay.**

<table>
<thead>
<tr>
<th>Well ID.</th>
<th>OD 620 nm Absorbance</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Readings</td>
<td>Average</td>
</tr>
<tr>
<td>Std-1: 0 µg/g (0 ng/mL)</td>
<td>0.043, 0.041</td>
<td>0.042</td>
</tr>
<tr>
<td>Std-3: 56.2 µg/g (156 ng/mL)</td>
<td>0.132, 0.120</td>
<td>0.126</td>
</tr>
<tr>
<td>Std-4: 145 µg/g (403 ng/mL)</td>
<td>0.494, 0.420</td>
<td>0.457</td>
</tr>
<tr>
<td>Std-5: 321 µg/g (892 ng/mL)</td>
<td>1.368, 1.380</td>
<td>1.374</td>
</tr>
<tr>
<td>Std-6: 669 µg/g (1860 ng/mL)</td>
<td>1.945, 1.950</td>
<td>1.948</td>
</tr>
<tr>
<td>Std-7: 2000 µg/g (5560 ng/mL)</td>
<td>2.415, 2.448</td>
<td>2.432</td>
</tr>
<tr>
<td>Control 2</td>
<td>1.145, 1.149</td>
<td>1.147</td>
</tr>
<tr>
<td>Control 3</td>
<td>1.778, 1.779</td>
<td>1.779</td>
</tr>
</tbody>
</table>

Control 2: 266.3 µg/g (740 ng/mL)
Control 3: 423.1 µg/g (1176 ng/mL)
EXPECTED VALUES

Stool samples from normal healthy adults with age of 24 – 58 were collected and measured with this ELISA. The recommended normal cut-off for fecal Calprotectin concentration by using this ELISA and sample collection system is 120 ng/mL or 43.2 µg/g directly read from assay standard curve. We strongly recommend that each clinical laboratory to establish its own normal cut-off level by measuring normal stool samples with this ELISA and sample collection system.

Please be aware that patients with recent diarrhea would give a much higher level of fecal Calprotectin. Taking spicy food or alcohol may also cause intestinal irritation resulting in an abnormal fecal Calprotectin level.

**Note:**  
Calprotectin ng/mL X 0.36 = Calprotectin µg/g  
Calprotectin µg/g X 2.78 = Calprotectin ng/mL

Please program ELISA reader by selecting assay standards concentration either in “µg/g” or “ng/mL to avoid manual calculation!

LIMITATION OF THE PROCEDURE
1. A strong positive of fecal calprotectin is likely to indicate a more significant clinical pathological condition of a patient. However, a low positive of fecal calprotectin does not indicate a lesser possibility of inflammation.
2. A normal fecal calprotectin level does not rule out the presence of any gastrointestinal diseases such as IBD.
3. For sample values reading greater than the highest standard, it is recommended to re-assay samples with dilution (i.e. 1:10 or 1:100 with Extraction Buffer).
4. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

QUALITY CONTROL
To assure the validity of the results each assay should include adequate controls.

PERFORMANCE CHARACTERISTICS

Sensitivity
The analytical sensitivity (LLOD) of the human Calprotectin ELISA Assay Kit as determined by the 95% confidence limit on 12 duplicate determination of zero standard is approximately 2.5 ng/mL. A LLOQ was determined by dilution of assay standards and it is about 5 ng/mL.

**High Dose “hook” effect**
This assay has showed that it did not have any high dose “hook” for calprotectin level up to 40,000 ng/mL in extraction buffer.

**Precision**
The intra-assay precision was validated by measuring three sample extracts in a single assay with 12 replicate determinations.

<table>
<thead>
<tr>
<th>Mean Calprotectin Value (µg/g)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.74</td>
<td>2.9</td>
</tr>
<tr>
<td>26.59</td>
<td>3.5</td>
</tr>
<tr>
<td>54.70</td>
<td>2.5</td>
</tr>
</tbody>
</table>

The inter-assay precision was validated by measuring two samples in duplicate in 4 individual assays.

<table>
<thead>
<tr>
<th>Mean Calprotectin Value (µg/g)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.64</td>
<td>8.6</td>
</tr>
<tr>
<td>70.31</td>
<td>2.0</td>
</tr>
</tbody>
</table>

The precision of inter-sample collection was performed by collecting five specimens from one bowel movement. These grouped samples are measured in an assay according to the assay procedure. The results of Calprotectin concentration in the value of ng/mL indicate that there are very satisfactory agreements of the five samples collected from one bowel movement.

<table>
<thead>
<tr>
<th>Donor</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>57.0</td>
<td>65.0</td>
<td>59.2</td>
<td>56.2</td>
<td>49.8</td>
<td>9.5</td>
</tr>
<tr>
<td>B</td>
<td>60.4</td>
<td>55.3</td>
<td>58.8</td>
<td>71.7</td>
<td>81.1</td>
<td>16.3</td>
</tr>
<tr>
<td>C</td>
<td>72.3</td>
<td>69.3</td>
<td>51.5</td>
<td>65.7</td>
<td>65.6</td>
<td>12.3</td>
</tr>
</tbody>
</table>
**Linearity**
One sample was diluted with assay buffer and tested. The results of Calprotectin concentration in the value of ng/mL are as follows:

<table>
<thead>
<tr>
<th>DILUTION</th>
<th>OBSERVED VALUE</th>
<th>EXPECTED VALUE</th>
<th>RECOVERY %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat</td>
<td>195.84</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:2</td>
<td>87.88</td>
<td>97.92</td>
<td>89.7</td>
</tr>
<tr>
<td>1:4</td>
<td>46.58</td>
<td>48.96</td>
<td>95.1</td>
</tr>
<tr>
<td>1:8</td>
<td>24.53</td>
<td>24.48</td>
<td>100.2</td>
</tr>
<tr>
<td>1:16</td>
<td>13.77</td>
<td>12.24</td>
<td>112.5</td>
</tr>
</tbody>
</table>

**Spike Recovery**
Three fecal extracts and three assay standards were spiked together in various volume combinations and tested. The results Calprotectin concentration in the value of ng/mL are as follows:

<table>
<thead>
<tr>
<th>#</th>
<th>Orig. Value</th>
<th>Amount Spiked</th>
<th>Observed Value</th>
<th>Expected Value</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30.0</td>
<td>37.1</td>
<td>61.9</td>
<td>67.1</td>
<td>92.2</td>
</tr>
<tr>
<td>2</td>
<td>73.0</td>
<td>12.7</td>
<td>85.7</td>
<td>89.3</td>
<td>104.2</td>
</tr>
<tr>
<td>3</td>
<td>217.7</td>
<td>30.3</td>
<td>248.0</td>
<td>256.9</td>
<td>96.5</td>
</tr>
</tbody>
</table>

**REFERENCES**
Calprotectin ELISA: Condensed Assay Protocol

1. 50 µl Assay Buffer per well
2. 50 µl Calibrators, controls and extracted patient samples
   \textit{Incubate @ RT for 60 min on ELISA plate shaker wash 5 x}
3. 100 µl Tracer Antibody
   \textit{Incubate @ RT for 45 min on ELISA plate shaker Wash 5 x}
4. 100 µl TMB Substrate
   \textit{Incubate @ RT for 12 min static}
5. Read absorbance at 620 nm
   \textit{Immediately}
6. 100 µl Stop Solution
7. Read absorbance at 450/650 nm
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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.