Fecal Mucin Assay Kit  
Cat. No. CSR-FFA-MU-K01E

**PURPOSE OF USE**
For determination of mucin content in feces

**BACKGROUND**
Mucins are a family of heavily glycosylated proteins, and main components of mucosa such as saliva, tear, gastric fluid enteric fluid. Basic configuration of mucin are macromolecules linked ramiform sugar chain to peptide framework. The heterogeneous property of sugar chain makes them diversity, the molecules has various function, such as specific molecular recognition. Some of the sugar chains recognize a specific protein derived from virus, bacteria. Mucins are positioned in mucosal barrier function in gut, stopping the translocation of pathogen and toxin into blood vessel beyond the intestinal wall.

**Assay principle**
Mucins are a family of high molecular (1000kda-10000kda) and heavily glycosylated protein. Mucin domains within the protein core are rich in threonine, serine and hydroxyproline. The reducing end of sugar chain (GalNAc) are freqeuncy-linked to these amino acid by the post-translational O-glycosylation.
This kit contains components to determine fecal mucin content.
Step1: Extraction and partial purification of mucin from feces.
Step2: Determination of mucin

O-glycosidically linked oligosaccharide chains is β-eliminated by diluted alkali, and reducing end of sugar chain is formed. Reducing carbohydrates react at high temperature with 2-cyanoacetamide (CAN) to produce intensity fluorescent condensate.

Kit components

<table>
<thead>
<tr>
<th>Components</th>
<th>Content</th>
<th>Quantity</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer A</td>
<td>Tablet for 100ml</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Buffer B (acetic acid)</td>
<td>25ml</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Buffer C (boric acid)</td>
<td>25ml</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Reagent A (2-Cyanoacetamide)</td>
<td>1.0ml</td>
<td>1</td>
<td>4〜10℃</td>
</tr>
<tr>
<td>Regent B (NaOH)</td>
<td>1.5ml</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Standard Solution (GalNAc 250μg/ml)</td>
<td>1.0ml</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Enzyme Solution</td>
<td>1.5ml</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
II. Assay protocol

Working Calibrator: N-acetylglucosamine 250μg/ml

- Create a standard curve by serial dilution as indicated in the table below.
- The remaining undiluted Standard Solution should be stored at 2-10°C.
- Diluted Calibrator is stable and should be stored at 2-10°C for 1 month.

<table>
<thead>
<tr>
<th>Calibrator Number</th>
<th>Quantity of Standard</th>
<th>Quantity of Dilution Buffer</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>500 μl of Standard Solution</td>
<td>0</td>
<td>250μg/ml</td>
</tr>
<tr>
<td>2</td>
<td>500 μl of Calibrator 1</td>
<td>500 μl</td>
<td>125μg/ml</td>
</tr>
<tr>
<td>3</td>
<td>500 μl of Calibrator 2</td>
<td>500 μl</td>
<td>63μg/ml</td>
</tr>
<tr>
<td>4</td>
<td>500 μl of Calibrator 3</td>
<td>500 μl</td>
<td>31.5μg/ml</td>
</tr>
<tr>
<td>5</td>
<td>500 μl of Calibrator 4</td>
<td>500 μl</td>
<td>15.75μg/ml</td>
</tr>
<tr>
<td>6</td>
<td>500 μl of Calibrator 5</td>
<td>500 μl</td>
<td>7.875μg/ml</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>500 μl</td>
<td>0</td>
</tr>
</tbody>
</table>

Preparation of Buffer A
Dissolve 1 tablet with 100ml of purified water.

Preparation of feces powder
Feces should be freeze-dried and grounded in a mortar and stored at -20°C until use.

II. Measurement of fecal mucin

1. Weigh 100 mg of feces powder into micro test tube, and add the 1ml of Buffer A, then mix the solution with vortex mixer for 30sec.
2. Heat the tubes at 95°C for 10 minutes to denature the glycosidase derived from bacteria.
3. Heat the tubes at 37°C for 90 minutes to extract the mucins from the feces.
4. Centrifuge 20,000×g at 4℃ for 15 minutes.
5. Transfer the 200ul of supernatant into another micro test tube, and add the Buffer B, then mix the solution with vortex mixer.
6. Add the 10ul of enzyme solution, mix together, and heat the tubes at 50℃ for 20 minutes to remove the dietary starch.
7. Cool down the tubes until room temperature, add 615ul of 99.5% ethanol. After mixing, settle the tubes at -20°C for overnight.
8. Next day, centrifuge the tubes 20,000×g at 4℃ for 10 minutes, remove the supernatant.
9. Add the 1ml of Buffer A to the precipitate, resolve the precipitate. (Sample solution)
10. Transfer the 20ul of sample solution and standard solution into a new micro test tube, and add the 24ul of 2-Cyanoacetoamid solution (mix together Reagent A and Reagent B, 1:5, just before use), after mixing, heat the tube up at 100°C for 30 minutes.
11. Cool down the tube until room temperature, add the 200ul of Buffer C, and mix together with vortex mixer.
12. Transfer the 100ul of the solution into the wells of 96 well black plate, and then measure the fluorescence using fluorescence plate reader set at a wavelength (ex:336nm em:383nm).
13. Create a standard curve by serial dilution as indicated in the below. Draw a smooth curve through these points to construct the calibration curve. Read the concentration for the samples from the calibration curve.

14. Calculation of mucin contents in the 1g of feces.
Value measured at step (12) times 50 is mucin contents in the 1g of feces.
Ⅲ. Example of Results

**Fig. 1 Standard Curve**

\[
y = -0.4526x^2 + 285.68x + 685.85
\]

\[R^2 = 0.9991\]

**Fig. 2 Measurement Example**

Dietary polyphenols derived from aronia, hascup and bilberry, markedly-elevated the amount of fecal mucin. (n=5)
IV. References

Fluorimetric Determination of Reducing Carbohydrates with 2-Cyanoacetamide and Application to

the invasive pathogen Salmonella enteritidis: additive effects of dietary lactulose and calcium. Gut

[3] Crowther RS, Wetmore RF: Fluorometric assay of O-linked glycoproteins by reaction with

of curcumin elevates fecal immunoglobulin A, an index of intestinal immune function, in rats fed a

Consumption of a Resistant Protein, Sericin, Elevates Fecal Immunoglobulin A, Mucins, and Cecal
10.3945/jn.111.144246.

Modulates Fecal Secondary Bile Acids, Mucins, Immunoglobulin A, Enzyme Activities, and Cecal

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