COSMO BIO CO., LTD.

Fecal Mucin Assay Kit

Cat. No. CSR-FFA-MU-K01E

Edition Date : 2016/08/23

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【I - 1.】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.

Fig.1 Structure of Mucin

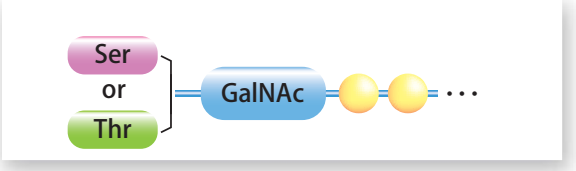
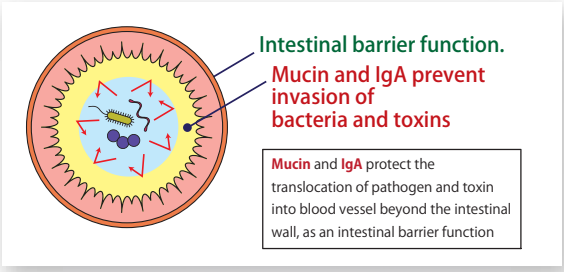


Fig. 2 Intestinal tract barrier function by mucin



【I - 2.】Features

Fecal Mucin Assay Kit is an easy to detection system for content of mucin in feces.

There is a possibility that it can be used for....

- Development of functional food
- Research of intestinal flora
- Food research
- Agricultural research

For research use only, Not for diagnostic use.
Please read this manual thoroughly before use.

【I - 3.】Assay principle

Mucins are a family of high molecular (1000 kda-10000 kda) 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 frequentry-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 are fluorescence-labeled at high temperature to produce intensity fluorescent condensate.

【I - 4.】Kit components

No.	Component	Volume	Quantity	Storage
1	Buffer A	Tablet for 100mL	3	4-10℃
2	Buffer B	25 mL	1	
3	Buffer C	25 mL	1	
4	Reagent A	1.0 mL	1	
5	Reagent B	1.5 mL	2	
6	Standard Solution（GalNAc 250 µg/mL）	1.0 mL	1	
7	Enzyme Solution	1.5 mL	1	

This product is designed for the measurement in fluorescence plate readers (96 well plates).
When using Fluorescence plate reader, it can measure 100 samples.
When you measure with a light spectrophotometer, please use it with a microcell.

Required but not provided

- Purified water
- 99.5% ethanol
- Fluorescent microplate reader and black plate
- Micro test tube（2 mL, 1.5 mL）
- Micro test tube（2 mL, 1.5 mL）
  (When you measure with a light spectrophotometer, please prepare a microcell)

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【II -1.】Assay protocol

Working Calibrator: N-acetylgalactosamine 250 µg/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℃.
- Diluted Calibrator is stable and should be stored at 2-10℃ for 1 month.

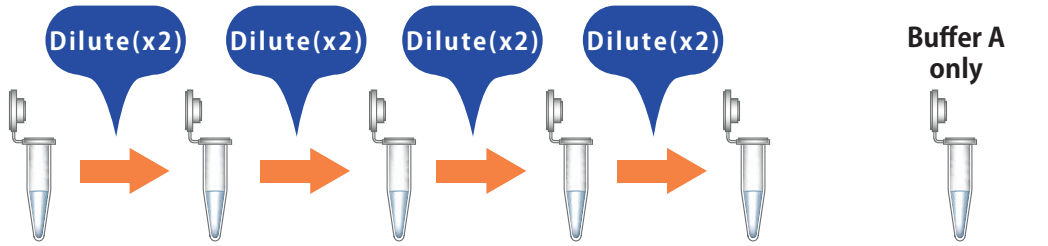
Preparation of buffer A

Dissolve 1 tablet with 100 mL of purified water.

Preparation of feces powder

Feces should be freeze-dried and grounded in a mortar and stored at -20℃ until use.

Preparation of standard solution



Conc.	① Standard 250 µg/mL	② 125 µg/mL	③ 62.5 µg/mL	④ 31.25 µg/mL	⑤ 15.625 µg/mL	⑥ Blank
Mixture						
Buffer A	-	500 µL	500 µL	500 µL	500 µL	500 µL
Standard A	-	Add①500 µL	Add②500 µL	Add③500 µL	Add④500 µL	0 µL

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【III-1.】Measurement of fecal mucin

1. Weight 100 mg of feces powder into micro test tube, and add the 1mL of Buffer A, then mix the solution with vortex mixer for 30 sec.
2. Heat the tubes at 95℃ for 10 minutes to denature the glycosidase derived from bacteria.
   Heat the tubes at 37℃ for 90 minutes to extract the mucins from the feces.
3. Centrifuge 20,000 × g at 4℃ for 15 minutes.
4. Transfer the 200 µL of supernatant into another micro test tube, and add 200 µL of Buffer B, then mix the solution with vortex mixer.
5. Add the 10 µL of enzyme solution, mix together, and heat the tubes at 50℃ for 20 minutes to remove the dietary starch.
6. Cool down the tubes until room temperature, add 615 µL of 99.5% ethanol. After mixing, settle the tubes at -20℃ for overnight.
7. Next day, centrifuge the tubes 20,000 × g at 4℃ for 10 minutes, remove the supernatant.
8. Add the 1 mL of Buffer A to the precipitate, resolve the precipitate. (Sample solution).
9. Transfer the 20 µL of each sample or standard solution (①～⑥) into the another micro test tube (for 500 µL). Add the 24 µL of reagent mixture (mix together Reagent A and Reagent B, 1:5, just before use) to the test tubes. After mixing, heat the tube up at 100℃ for 30 minutes.
10. Cool down the tube until room temperature, add the 200 µL of Buffer C, and mix together with vortex mixer.
11. Transfer the 100 µL of the solution into the wells of 96 well black plate, and then measure the fluorescence using florescence plate reader set at a wavelength (ex: 336 nm em: 383 nm).
12. 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.
    CalcµLation of mucin contents in the 1 g of feces.
13. Value measured at step (12) times 50 is mucin contents in the 1g of feces.

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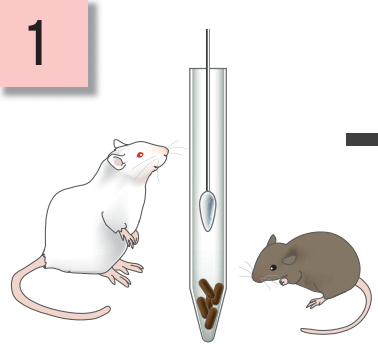
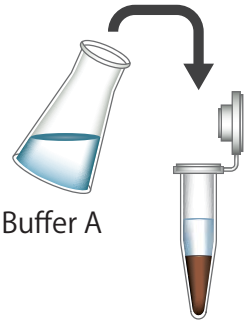
【 III-2. 】 Measurement -flow chart- step 1~6: 3~4 hrs

1



Rat or Mouse
Feces should be
freeze-dried and grounded



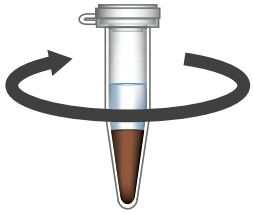
- Weigh 100 mg of feces powder into **2.0 mL micro test tube**.
- Add the 1 mL of Buffer A、mix the solution with vortex mixer

2

Heat the tubes at 95℃ for 10 minutes

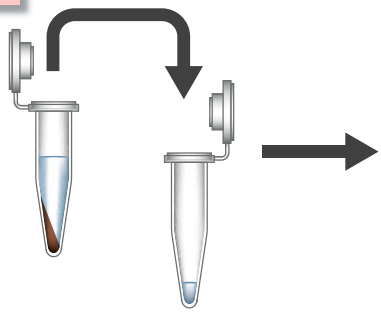
Heat the tubes at 37℃ for 90 minutes

3

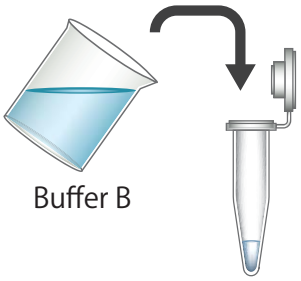


Centrifuge 20,000×g at 4℃ for 15 minutes

4

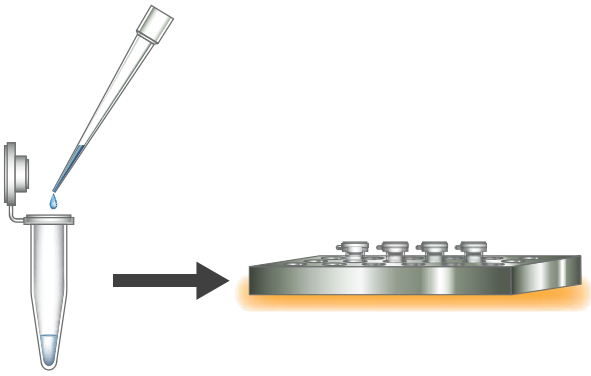


Transfer the 200 µL of supernatant into
new micro 1.5 mL test tube



Add the 200 µL of Buffer B, then mix the solution with vortex mixer

5

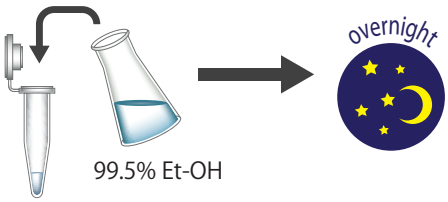


Add the 10 µL of enzyme solution

Heat the tubes at 50℃ for 20 minutes

6

- Cool down the tubes until room temperature
- Add 615 µL of 99.5% ethanol and mix
  Settle the tubes at -20℃ for overnight



99.5% Et-OH

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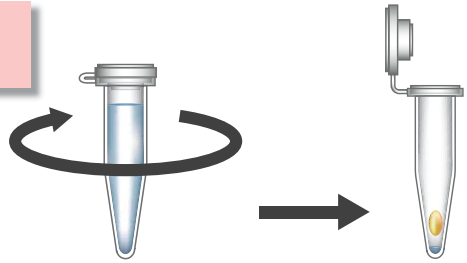
【III-3.】Measurement -flow chart- continued step 7~13: 1~1.5 hrs

7

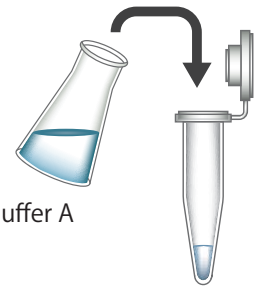


20,000×g、4℃、10 min
Centrifuge the tubes

Remove the supernatant

Precipitate can be stored at -20℃

8



Add the 1 mL of Buffer A to the precipitate
Mix well and resolve the precipitate

☆This solution becomes sample solution

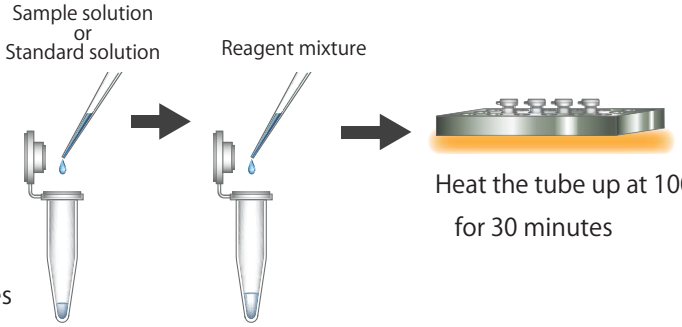
9

- Mix together Reagent A and Reagent B, 1: 5, just before use

☆This solution becomes reagent mixture

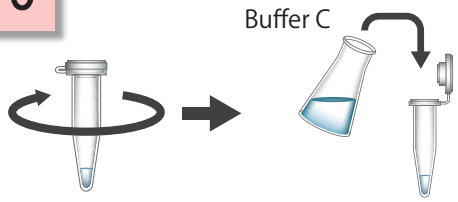
- Sample solution ： 20 µL
  or, Standard solution ： 20 µL
- Reagent mixture ： 24 µL

Move these reagents to new 500 µL tubes



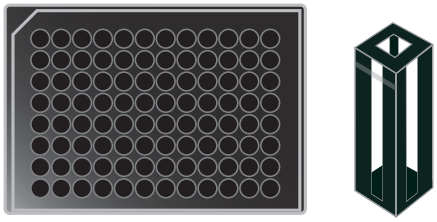
Heat the tube up at 100℃
for 30 minutes

10



Cool down the tube until room temperature, add the 200 µL of Buffer C, and mix together with
vortex mixer

11

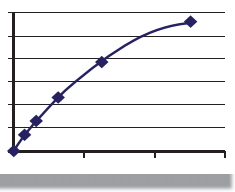


Transfer the 100 µL of the solution into the wells of 96 well black plate, and then measure the fluorescence using florescence plate reader set at a wavelength (ex:336 nm em:383 nm)

12

Create a standard curve by serial dilution as indicated in the below.

Draw a smooth curve through these points to construct the calibration curve



13

Value measured at step (12) times 50 is mucin contents in the 1 g of feces

Mucin

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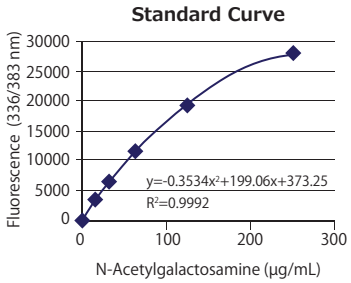
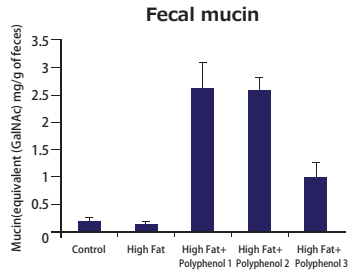
【IV.】Example of results

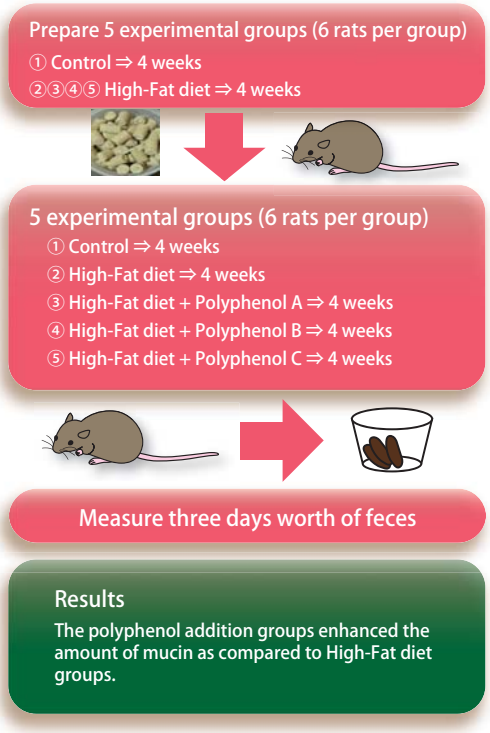
Effects of polyphenols administration on the intestinal barrier function in high-fat diet fed rats.



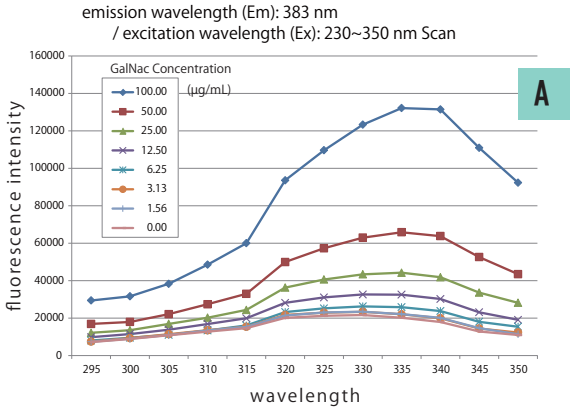


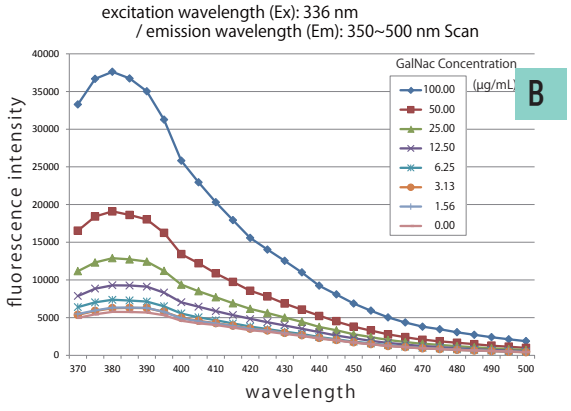
Measurement Example

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



【V】Reference data





Changes of excitation wavelength and fluorescent wavelength in standard solution of each density

A : Excitation spectrum for Emission wavelength (Em) 383 nm
B : Emission spectrum for Excitation wavelength (Ex) 336 nm

The recommended measurement wavelengths are 336 nm for Ex and 383 nm for Em, but you might not be able to measure the fluorescence wavelength when fluorescence plate reader with interference filter system is used. In such a case, please shift the fluorescent wavelength to longer.

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【VI.】References

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[5] Yukako Okazaki,Hiroyuki Tomotake, Kazuhisa Tsujimoto, Masahiro Sasaki,and Norihisa Kato. Consumption of a Resistant Protein, Sericin, Elevates Fecal Immunoglobulin A, Mucins, and Cecal Organic Acids in Rats Fed a High-Fat Diet. (2011) The Journal of Nutrition,21, 10.3945/jn.111.144246.

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