COSMO BIO CO., LTD.

Tear Mucin Assay Kit (O-Glycan Assay Method)

Cat. No. CSR-MUC01E

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【I - 1】Background

Mucins are major components in tear fluid and apical cell membranes on the ocular surface epithelia. Structurally, they are composed of tandem repeat domains containing heavily O-glycosylated serine and threonine residues. More than a half of its weight consists of O-glycans, which has hydrophilic nature (Fig. 1). The heavy glycosylation of mucins is believed to impart a highly negative charge and a hydrophilicity that provides a barrier to pathogen adherence and penetrance into the epithelium (Fig. 2). Alteration in both secreted and membrane-associated mucins occur in drying ocular surface diseases (Fig. 2). At the ocular surface, three types of mucins are present. The large gel-forming mucin MUC5AC is expressed by conjunctival goblet cells. Some cells of the lacrimal gland acini express the small soluble mucin MUC7. The corneal and conjunctival epithelia express the membrane-associated mucins MUCs 1, 4, and 16 (Fig. 2).

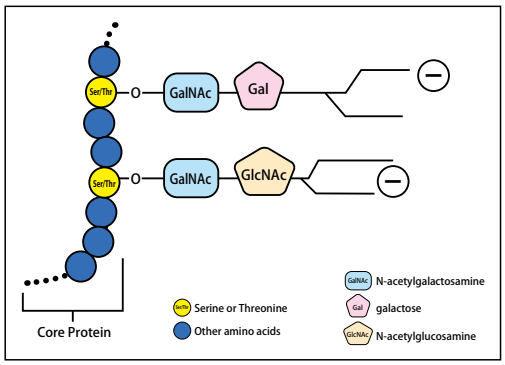
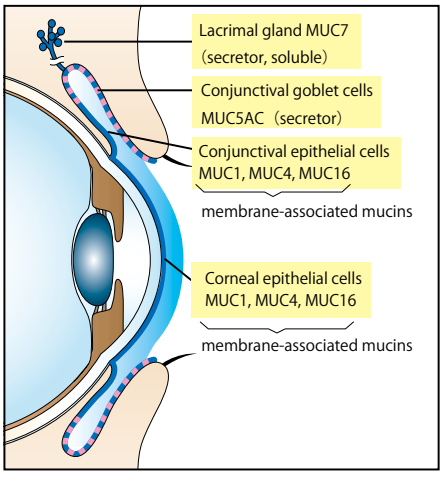


Fig. 1 Structure of mucin



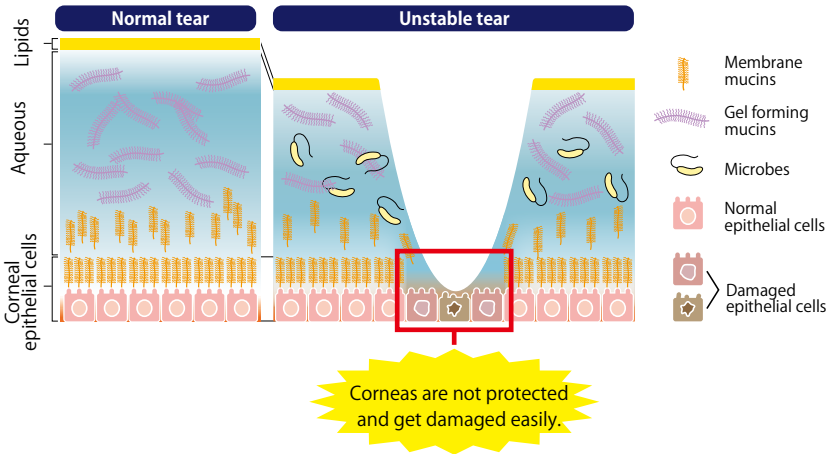


Fig. 2 Mucins expressed around corneal surface, and barrier function by tear mucins

【Ⅰ - 2】Assay Principle

Mucins are family of high molecular (1000 kda-10000 kda) and heavily glycosylated protein. Mucin domains within the protein core are rich in threonine and serine. The reducing ends of sugar chain N-acetylgalactosamin (GalNAc) are linked to those amino acids by the post-translational O-glicosylation (Fig.1). Mucin content can be measured as reducing ends of sugar chain after β -eliminated by diluted alkali. Reducing ends of sugar chain react at high temperatures with 2-cyanoacetamide (2-CAN) to produce intensely fluorescent condensate.

【Ⅰ - 3】Kit Components

No.	Component	Volume	Quantity	Strage
1	Elution Buffer	30 mL	1	4-10℃
2	Slurry for Spin Column	45 mL	1	
3	Standard Solution (GalNAc 100 µg/mL)	1.0 mL	1	
4	Reagent A	0.3 mL	1	
5	Reagent B	1.5 mL	1	
6	Stop Solution	15 mL	1	
7	Empty column	For 1mL	50	
8	Centrifuge tube	-	50	

This kit contains all the components to determine the mucin contents in tear fluid.

Required but not provided:

- Filter Paper (Schirmer strips)
- Micro test tube (2.0 mL, 1.5 mL, 0.5 mL)
- Fluorescent microplate reader and black plate

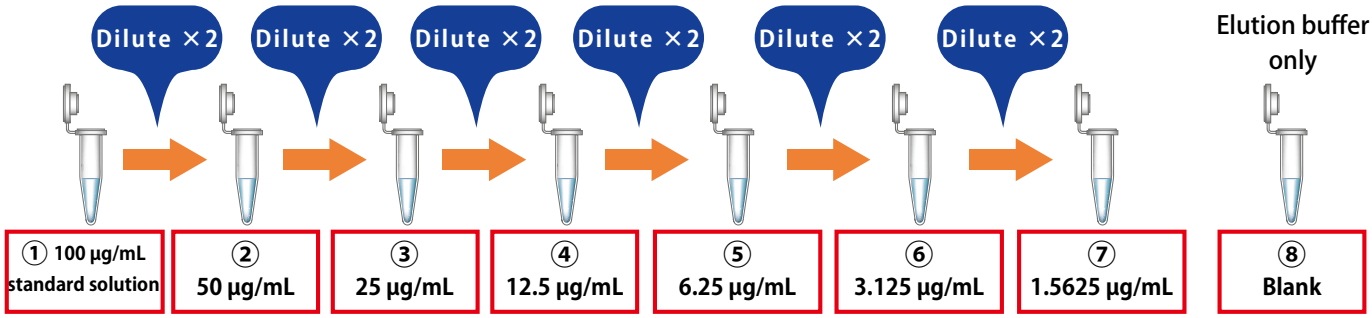
(Please use a microcell when you measure with a fluorescent spectrophotometer.)

【Ⅱ - 1】Assay Protocol

Working Calibrator : N-acetylgalactosamin (GalNAc)100 µg/mL

- Create a standard curve by serial dilution as indicated in the table below.
- The remaining undiluted Standard Solution can be stored at 2-10℃ for 1 year.
- Diluted Calibrator is stable and can be stored at 2-10℃ for 3 weeks.

	①	②	③	④	⑤	⑥	⑦	⑧
Conc.	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.5625 µg/mL	Blank
Elution Buffer	-	500µL	500µL	500µL	500µL	500µL	500µL	500µL
	Standard Solution	Add ① 500µL	Add ② 500µL	Add ③ 500µL	Add ④ 500µL	Add ⑤ 500µL	Add ⑥ 500µL	-



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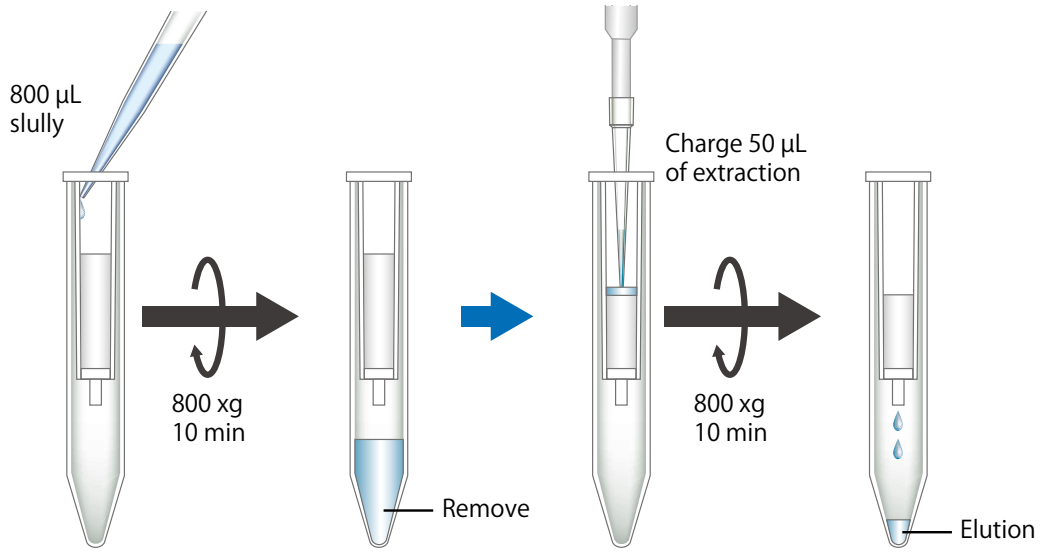
【II - 2】Sample Preparation

- Collect a tear fluid for 5 minutes using Schirmer strips.
- If you store the filter paper collected tear fluid, please put it in micro test tube and store it at 4℃ . Avoid freezing.

【III】Measurement of tear mucin

1. Transfer the Schirmer strips into micro test tube and add 200 µL of Elution Buffer, and extract mucin for 1 hour at room temperature.
2. During 1 hour extraction of step 1, prepare empty column. Set the empty column into centrifuge tube. Agitate the Slurry bottle, until uniformalized. Add 800 µL of uniformalized slurry into the empty column. Centrifuge the column for 10 minutes at 800 x g at room temperature. (Recommended equipment is a centrifugal of the swing rotor system.) Remove the pass though liquid in the bottom of centrifugal tube.
3. Charge 50 µL of the extraction from Schirmer strips prepared at step 1 onto the upper part of the packed gel of step 2.
4. Centrifuge the sample charged columns for 10 minutes at 800 x g at room temperature. Collect the pass through fraction (approximately 50 µL).



5. Transfer the 20 uL of each pass through fraction (4) or standard solution(①～⑧) into the another micro test tube (for 500 uL). Add 24 uL of reagent mixture (mix together Reagent A and Reagent B, 1：5 (v/v), just before to use) to the test tube. After mixing, heat the tube up to 100℃ for 30 minutes.
6. Cool down the tube until room temperature, add the 200 uL of stop solution, and mix together with vortex mixer.
7. Transfer the 100 uL of the solution into the wells of 96 well black plate, and then measure the fluorescence using fluorescence plate reader set at wavelength (Exitation:336 nm, Emission：383 nm).
8. 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 of the sample from the calibration curve.

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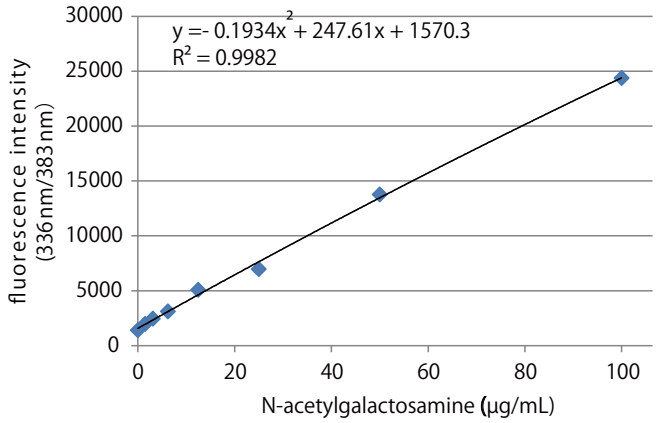
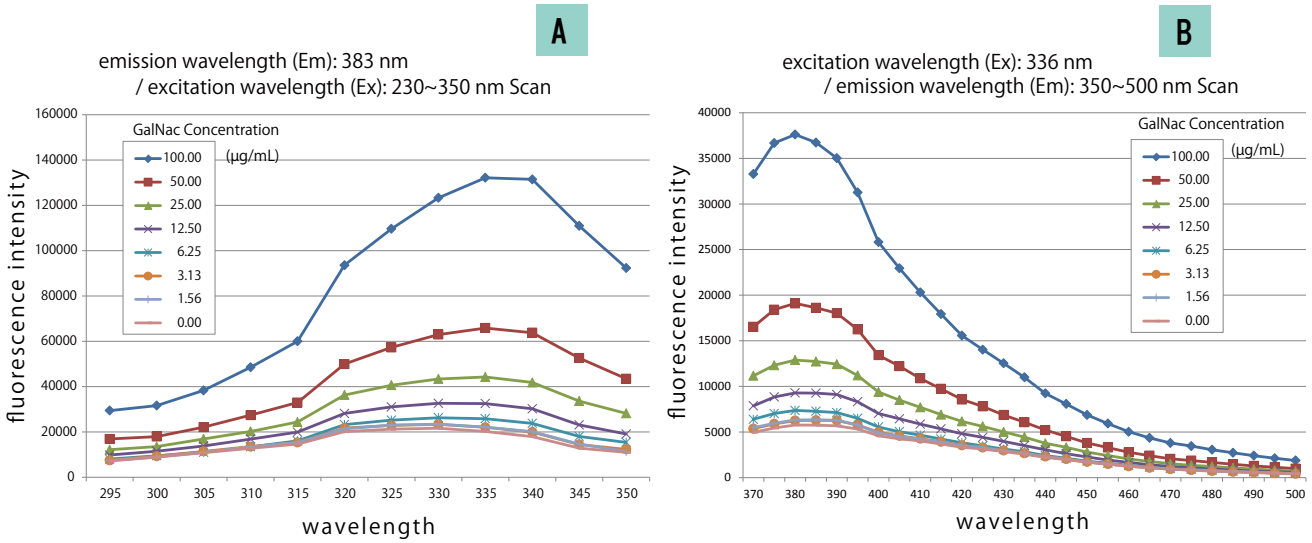
【IV】Standard Curve



【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 leader 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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