

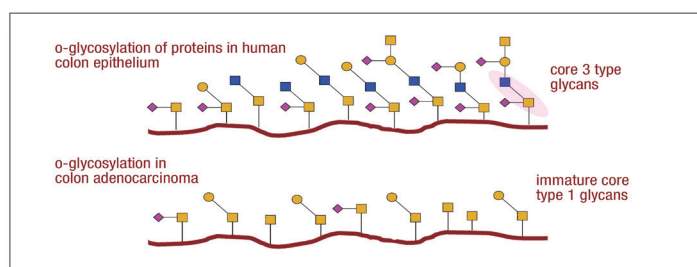
Enzymatic deglycosylation of a protein containing Core 1 O-glycans with O-Glycosidase

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For many cancers, such as colon, ovary, uterus and bladder (mucosas), tumor progression and its poor prognosis strongly correlates with alterations in the patterns of mucin O-glycosylation. For instance, β 3Gn-T6 (the enzyme responsible for Core 3 O-glycan synthesis), is abundant in normal colon tissue while its expression is strongly downregulated in adenocarcinoma (1,2,3). As a result, mucin glycosylation switches from common Core 3 O-glycan structures to short Core 1 structures. These Core 1 structures, T and Tn, are hallmark epitopes of cell malignancy (4).

The study of glycan aberrations in cancer opens new avenues in the development of novel therapies. In this regard, the endo-O-glycosidase is proving useful to characterize and quantitate a variety of commonly found O-glycan forms.

This application note describes the use of a recombinant O-Glycosidase that can cleave core 1 O-linked disaccharides (5) and immature Tn core (GalNAc). Conditions have been determined that allow this O-Glycosidase to be used under the same denaturing conditions used for PNGase F digestion.



General Protocol

1. Set up the reaction in 200 μ l tubes as follows:

COMPONENTS	DENATURING CONDITIONS	NATIVE CONDITIONS
Fetuin (10 mg/ml) in water	18 μ l	18 μ l
10X Denaturing Buffer	2 μ l	2 μ l
Incubate 95°C for 5 minutes. Cool on ice, spin		
10X NP-40	4 μ l	–
10X GlycoBuffer 2	4 μ l	4 μ l
Milli-Q Water	18 μ l	24 μ l
Neuraminidase	2 μ l	2 μ l
O-Glycosidase	2 μ l	2 μ l

- Incubate at 37°C for 1 to 4 hours. After incubation, add 1 μ l of 4M KCl followed by 150 μ l of methanol. Chill overnight at 4°C to precipitate proteins. After the overnight precipitation, spin the sample at 14,000 rpm for 30 minutes, and reserve the supernatant.
- Concentrate supernatant to dryness with a Speed Vac set at medium heat (Savant; equipped with a high vacuum pump and finger trap immersed in a Dewar containing isopropanol and dry ice). Reconstitute with 400 μ l Milli-Q® water.

(see other side)

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Materials

- O-Glycosidase & Neuraminidase Bundle (NEB #E0540)
- Fetuin (NEB #P6042)
- Core 1 disaccharide (Gal β 1,3GalNAc; Accurate Chemical #BCR20/06)

- De-ionize the sample from step 4 by gently rocking in 200 µl of prepared mixed bed ion exchange resin AG 501-X8 (Bio-Rad; #142-6424) for 5 minutes. Collect the supernatant with a 1 ml syringe using a 23 gauge needle. **Note:** before use, the resin must be converted to the acetate form by soaking in an equal volume of 1 M acetic acid followed by washing ten times with equal volumes of water.
- Remove the needle and load the entire sample (400 µl) from Step 5 to an activated Sep-Pak® cartridge (Waters; #WAT051910). Collect the entire flow through (400 µl). Wash the Sep-Pak 2 times with 400 µl of Milli-Q water and pool the washes with the flow through. Concentrate to 70 µl using a Speed Vac. **Note:** before use, the Sep-Paks are activated by washing two times with 400 µl methanol followed by 4 times with 400 µl Milli-Q water.
- Detect free sugars by HPAEC-PAD Chromatography using the following conditions:
Column: CarboPac 20 with Amino Guard.
Elution: 40 mM NaOH isocratic for 12 minutes, 150 mM regeneration for 10 minutes, flow rate: 0.5 µl/min.
Detection: Pulse electrochemical, Au electrode, quadruple potential.
Injection sample: 30 µl, with or without internal Galβ1,3GalNAc standard (30 nanograms).

References

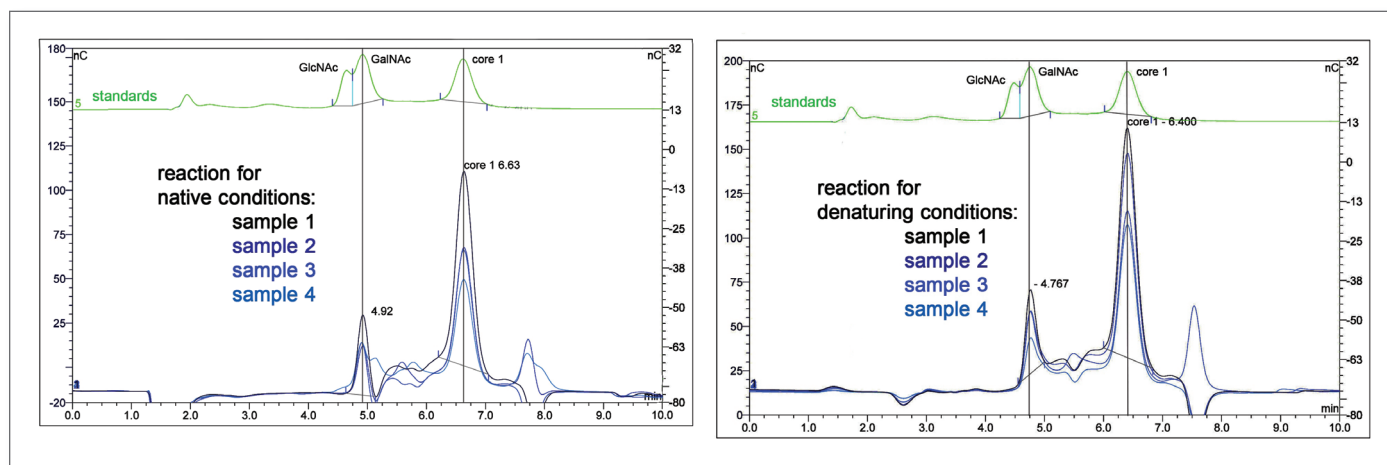
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Results:

Figure 1. Superimposed chromatograms of released sugars



Decreasing amounts of enzyme were used, as shown in table below. The chromatograms represent a 5-fold increase of all materials in step 2.

Sample #	units	O-GLYCOSIDASE ON NATIVE FETUIN		O-GLYCOSIDASE ON DENATURED FETUIN	
		GalNAc Peak Area nC*min	Core 1 Peak Area nC*min	GalNAc Peak Area nC*min	Core 1 Peak Area nC*min
1	200,000	9.55	36.92	9.84	43.29
2	10,000	6.23	21.85	8.02	39.91
3	50,000	6.74	23.01	8.07	27.12
4	12,500	4.51	18.08	5.09	25.11

