Removal of terminal galactose from a glycoprotein containing tri- and tetra-antennary *N*-linked sugars with α1-3, 6 Galactosidase

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With advances in transplantation and stem cell research, there has been a renewed interest in the study of glycoforms carrying the Galα1-3Gal epitope.

This motif is widely present in non-primate mammalian cells, while absent in Old World monkeys and humans (1). Naturally occurring high levels of anti-Gal antibodies cause xenotransplantations to fail within a few hours (2). This ability to ablate Gal-exposing cells has been exploited to develop safer human tissue grafts (3).

Specific glycosidases are required to characterize these kinds of systems. This application note describes the use of an α1-3,6 Galactosidase from *Xanthomonas manihotis* (recombinant expressed in *E. coli*) to remove terminal galactose residues from the tri- and tetra- antennary *N*-glycoprotein Bovine Thyroglobulin (4).

Materials

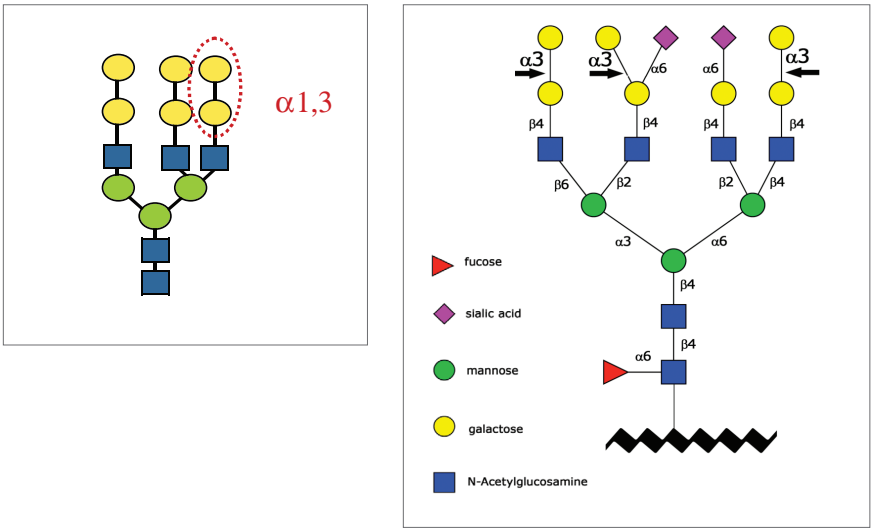
α1-3,6-Galactosidase
(NEB #P0731)

Galactose standard
(Sigma #G0750)

Bovine Thyroglobulin (Calbiochem; #609310)

10X G6 buffer
(supplied with enzyme)

Structure of the Bovine Thyroglobulin tetra-antennary carbohydrate moiety. Arrows denote the α1-3,6 Galactosidase cleavage sites.



General Protocol

1. Preparation of Glycoprotein substrate: Dialyze 1 µl of a 10 mg/ml solution of Bovine Thyroglobulin in water against 100 volumes of G6 buffer, for 4 hours at 4°C. The dialyzed solution can be stored in aliquots of 100 µl.

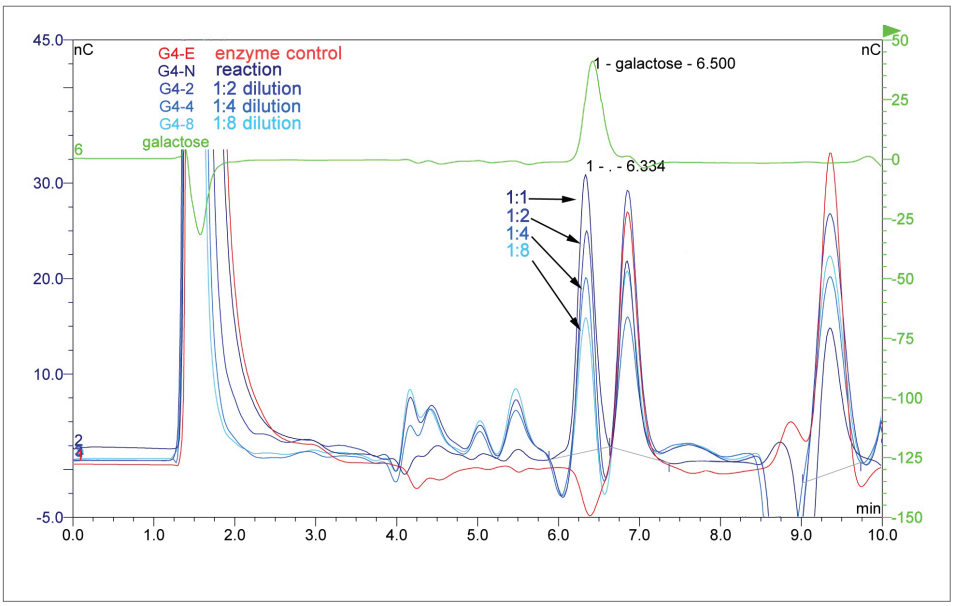
Glycoprotein Substrate 10 mg/µl	85 µl
G6 Buffer (10X)	10 µl
α1-3,6 Galactosidase	5 µl (20 units)
Total volume	100 µl

2. Incubate at 37°C for 4 hours. Add 200 µl water followed by 600 µl methanol (1)*. Chill overnight at 4°C to precipitate proteins. After the overnight precipitation, spin the sample at 14 K rpm for 30 minutes, and reserve the supernatant.

3. 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 µl Milli-Q™ water.
4. De-ionize the sample from step 4 by gently rocking in 200 µl of prepared mixed bed ion exchange resin AG 501-X8 for 5 minutes (Bio-Rad; #142-6424). Collect the supernatant with a 1ml 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.
5. 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.
6. Detect free galactose by HPAEC-PAD Chromatography using the following conditions: Column: CarboPac 20 with Amino Guard. Elution: 20mM 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 Galactose standard (30 nanograms).

Results:

FIGURE 1: **Superimposed chromatograph of released sugars.**
Chromatogram showing galactose peak released by serial decreasing amounts of a1-3,6 Galactosidase for the same amount of substrate. The superimposed peaks are designated 1:1 (20 units); 1:2 (10 units), 1:4 (5 units) and 1:8 (0.5 units).



References

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3. Hewitt, Z., et al., (2007) *Stem Cells* 25 10–18.
4. Wong-Madden, T., et. al., (1995) *Glycobiology* 5, 19–28.