α1-3,6 Galactosidase Application Note

Removal of terminal galactose from a glycoprotein containing tri- and tetra-antennary *N*-linked sugars.

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 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).

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.

2. Set up the Degalactosylation reaction:

Glycoprotein Substrate 10 mg/µl	85 µl
3. G6 buffer (10X)	10 µl
4. Xanthomonas α1-3,6 galactosidase	5 µl (20 units)
5. Total volume	100 µl

6. 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.

7. 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.

8. 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.

9. 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.

(see other side)

Materials

- α1-3,6-Galactosidase (NEB #P0731)
- Galactose standard (Sigma #G0750)
- Bovine Thyroglobulin (Calbiochem; #609310)
- 10X G6 buffer (supplied with enzyme)

**Alternatively, de-galactosylated proteins can be isolated and purified from free sugars using a centrifuge filter device.*

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10. Detect free galactose by HPAEC-PAD Chromatography using the following conditions: Column: CarboPac 20 with Amino Guard. *Elution:* 20mM NaOH isocratic for 12 minutes, 150mM 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).

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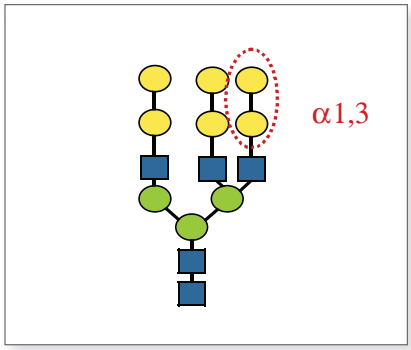
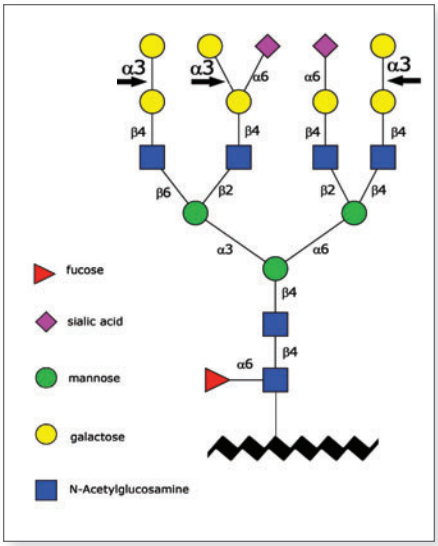
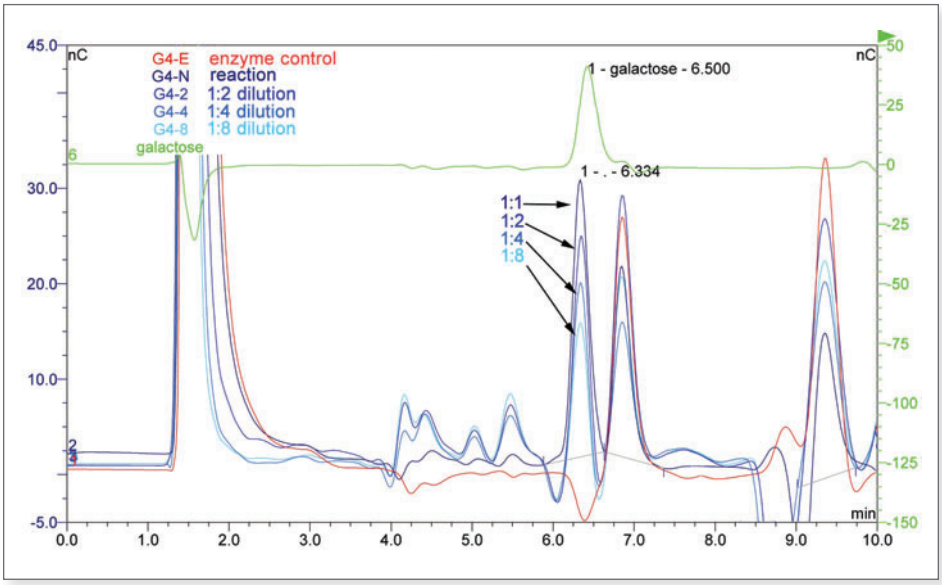
References

1. Koike, C. et al., (2002) *The Journal of Biological Chemistry* 227, 10114-10120.
2. Cooper, D.K., et al., (1993) *Lancet* 342, 682-683.
3. Hewitt, Z., et al., (2007) *Stem Cells* 25 10-18.
4. Wong-Madden, T., et. al., (1995) *Glycobiology* 5, 19-28.



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





Chromatogram showing galactose peak released by serial decreasing amounts of α1-3,6 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).

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