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Removal of terminal galactose from a glycoprotein containing tri- and tetraantennary *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 Gal α 1-3Gal 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 α 1-3,6 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).

Structure of the Bovine Thyroglobulin tetra-antennary carbohydrate moiety. Arrows denote the α 1-3,6 Galactosidase cleavage sites.





General Protocol

1. Preparation of Glycoprotein substrate: Dialyze 1 μ l 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 μ l.

| Glycoprotein Substrate 10 mg/µl | 85 µl |
|---------------------------------|-----------------|
| G6 Buffer (10X) | 10 µl |
| α 1-3,6 Galactosidase | 5 µl (20 units) |
| Total volume | 100 µl |

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.

Materials

 $\substack{\alpha$1-3,6-Galactosidase\\(\text{NEB \#P0731})}$

Galactose standard (Sigma #G0750)

Bovine Thyroglobulin (Calbiochem; #609310)

10X G6 buffer (supplied with enzyme)

- 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 μ l 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.
- 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).



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