

# Selectin Biosciences Inc.

## $\alpha$ (1-3,6) Galactosidase

$\alpha$ (1-3,6) Galactosidase ( $\alpha$ -D-galactoside galactohydrolase, EC 3.2.1.22) cleaves  $\alpha$ (1-3)- and  $\alpha$ (1-6)-linked, non-reducing terminal galactose from complex carbohydrates and glycoproteins. There is no activity on  $\alpha$ (1-4)-linked galactose. It is particularly efficient for removing  $\alpha$ -linked galactose under conditions where the pH must be neutral or above, for example, with living cells.

$\alpha$ (1-3,6) Galactosidase is useful for:

- Structural analysis of oligosaccharides
- Xenograft transplantation studies
- Removing heterogeneity from glycoproteins

$\alpha$ (1-3,6) Galactosidase is isolated from a strain of *E. coli* expressing a cloned gene from *E. coli*.

**Product Code:** GE 81

### Specifications

**Activity:** 30 U/mg, 400 U/mL

**Storage:** Store at 4°C. Do not freeze.

**Formulation:** The enzyme is provided as a sterile-filtered solution in 50 mM sodium phosphate pH 7.5.

**Stability:** Stable at least 12 months when stored properly. Several days exposure to ambient temperature will not reduce activity.

### Product Description

**Molecular weight:** ~80,000 Daltons

**Purity:**  $\alpha$ (1-3,6) galactosidase is tested for contaminating protease as follows; 10  $\mu$ g of denatured BSA is incubated for 24 hours at 37°C with 2  $\mu$ L of enzyme. SDS-PAGE analysis of the treated BSA shows no evidence of degradation.

The production host strain has been extensively tested and does not produce any detectable glycosidases.

**Specificity:** Non-reducing terminal  $\alpha$ (1-3)- and  $\alpha$ (1-6)-linked galactose. There is no activity on  $\alpha$ (1-4)-linked galactose.

### Assay

One unit of  $\alpha$ (1-3,6) galactosidase activity is defined as the amount of enzyme required to produce 1  $\mu$ mole of *p*-nitrophenol (*p*NP) in 1 minute at 25°C, pH 6.5 from *p*-nitrophenyl- $\alpha$ -D-galactopyranoside.

### Reagents:

- 5X Reaction Buffer 6.5 - 250 mM sodium phosphate pH 6.

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## Suggestions for Use

### Procedure For De-galactosylation

1. Add up to 100 µg of glycoprotein or 1 nmol of oligosaccharide to tube.
2. Add deionized water to a total of 14 µL
3. Add 4 µL of 5X Reaction Buffer 6.5.
4. Add 2 µL  $\alpha(1-3,6)$  galactosidase.
5. Incubate at 37°C for 1 hour. Longer incubations are necessary if fucose is present on the penultimate sugar.

## References

1. Kobata, A. Use of endo- and exoglycosidases for structural studies of glycoconjugates. **Anal Biochem** **100**: 1-14 (1979).
2. Prime, S. J. Dearnley, A.M. Venton, R.B. Parekh and C.J. Edge. Oligosaccharide sequencing based on exo- and endoglycosidase digestion and liquid chromatographic analysis of the products. **J Chromatogr A** **720**: 263-274 (1996)
3. Dwek, R.A. , C.J. Edge, D.J. Harvey, M.R. Wormald and R.B. Parekh. Analysis of glycoprotein-associated oligosaccharides. **Ann Rev Biochem** **62**: 65-100.

4. Schmid K, Schmitt R Raffinose metabolism in Escherichia coli K12. Purification and properties of a new alpha-galactosidase specified by a transmissible plasmid **Eur J Biochem** **67**(1):95-104 (1976)

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