



## $\beta$ (1-4)-Galactosidase

### Source

Recombinant from *Streptococcus pneumoniae* in *E.coli*

### Catalog Number

E-BG07	60 $\mu$ l
E-BG07-200	200 $\mu$ l
E-BG07-20	20 $\mu$ l

### Recommended Reagents

included with E-BG07:

1 vial: Reaction buffer – 250mM Sodium phosphate, pH 6.0

**Activity**  $\geq 3$  U/ml

**Specific Activity**  $\geq 6$  U/mg

**Molecular Weight** ~350,000 dalton

**pH optimum** 6.0, active over the range 5-7.

The supplied buffer concentrate provides the optimal pH for enzyme activity with the standard substrate. If glycosidase treatment is performed at suboptimal pH because of glycoprotein solubility or activity requirements, expect some diminution in enzyme activity.

### Formulation

The enzyme is provided as a sterile-filtered solution in 20 mM Tris-HCl, 25 mM NaCl (pH 7.5).

### Specific Activity

One unit of  $\beta$ -(1-4)-galactosidase is defined as the amount of enzyme required to produce 1  $\mu$ mole of p-nitrophenol (pNP) in 1 minute at 37°C pH 5 from p-nitrophenyl-beta-D-galactopyranoside.

### Specificity

Non-reducing terminal  $\beta$ (1-4)-Galactose. Number of antennae does not affect cleavage rate. Fucose linked to the penultimate N-acetylglucosamine will block cleavage of the galactose.

### Stability

Stable at least 12 months when stored properly. Several days exposure to ambient temperatures will not reduce activity.

### Storage

Store enzyme at 4°C. Do not freeze.

### Purity

$\beta$ (1-4)-Galactosidase is tested for contaminating protease as follows: 10  $\mu$ g of denatured BSA is incubated at 37°C for 24 hours 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.

## $\beta$ -(1-4)Galactosidase Specifications - Protocol

**Directions for use**

1. Add up to 100 µg of asialoglycoprotein 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 6.0.
4. Add 2 µl β(1-4) Galactosidase.
5. Incubate at 37°C for 1 hour.

For glycoproteins, cleavage may be monitored by SDS-PAGE if the size differential between native and de-galactosylated protein is sufficient for detection.

Note: The optimum pH for cleavage of oligosaccharides is ~6.

**References:**

Glasgow, L.R., J.C. Paulson and R.L. Hill. Systematic purification of five glycosidases from *Streptococcus pneumoniae*. **J Biol Chem** **252**: 8615-8623 (1977).

Kobata, A. Use of endo- and exoglycosidases for structural studies of glycoconjugates. **Anal Biochem** **100**: 1-14 (1979).

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)

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 (1993)

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This product is intended for *in vitro* research only.

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