

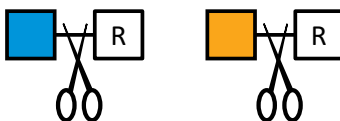
**$\beta$ -N-Acetylhexosaminidase contents**

Catalog #	Description	Size	M. W.	Purity	pH	Storage
GE1101	$\beta$ -N-Acetylhexosaminidase	500 units, lyophilized	51,020	> 95%	6.0-8.0 optimal	-20°C, up to 12 months
BA0801	10X Reaction Buffer 4	1 mL			7.5	4 to 25°C

*This product is for research use only and not for resale or for any use in the manufacture of a therapeutic or for any diagnostic purpose*

**Product description:** This product is recombinant  $\beta$ -N-Acetylhexosaminidase (glycosyl hydrolase family GH20; E.C. 3.2.1.52), cloned from *Streptococcus pneumoniae* and expressed in *Escherichia coli* with an N-terminal 8xHis tag. The 8xHis tag may be removed by digestion with FasTEV™ (Cat #GE0501), a TEV protease with enhanced stability and catalytic activity.

This product catalyzes the hydrolysis of the non-reducing terminal N-Acetylglucosamine (GlcNAc) and N-Acetylgalactosamine (GalNAc) from oligosaccharides and glycoprotein substrates.



This product does not contain any detectable activities of proteases or other glycosidases.

**Unit definition:** One unit is defined as the amount of enzyme required to catalyze the release of 1 nmole p-nitrophenol (pNP) from p-nitrophenyl-N-acetyl- $\beta$ -D-glucosaminide (pNP-GlcNAc) in 1 min at 37°C in 100  $\mu$ L 1X Reaction Buffer 4 (50 mM Tris-HCl, 100 mM NaCl, pH 7.5).

**Product reconstitution:** Dissolve the lyophilized product in 100  $\mu$ L molecular grade water to make a 5,000 units/mL (Cat #GE1101) solution in enzyme storage buffer (50 mM Tris-HCl, 100 mM NaCl, pH 7.5). Once reconstituted, store at 4°C for up to 7 days or -20°C for up to 3 months. Aliquoting is recommended to avoid repeated freeze-thaw cycles.

**Activity assay:** One unit of enzyme is added to 100  $\mu$ L of 500  $\mu$ M pNP-GlcNAc in 1X Reaction Buffer 4 at 37°C, followed by real-time measurements of absorption at 405 nm every 10 s for 120 s.

**Suggested protocol for removal of GlcNAc or GalNAc from oligosaccharides:**

- Mix the following components in a microfuge tube:
 

Oligosaccharide (e.g., NatGlycan Cat #NG-CM-012)	10 $\mu$ g
10X Reaction Buffer (Cat #BA0801)	10 $\mu$ L
$\beta$ -N-Acetylhexosaminidase (Cat #GE1101)	2.0 $\mu$ L (10 units)
Molecular grade water	to 100 $\mu$ L final volume
- Incubate at 37°C for 1 to 4 h.
- Analyze reaction products by mass spectrometry or other method to monitor the progress of the reaction. If a glycoprotein is used as the substrate, Western Blot may be used to determine the extent of reaction completion. Suggested 1° probes for Western blot analysis: GlcNAc or GalNAc-specific lectin (e.g., biotinylated GSL II).

**Reference:** Clarke VA, et al. J Biol Chem. 1995 Apr 14;270(15):8805-14. PMID: 7721787