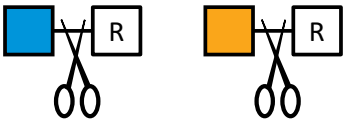
β-*N*-Acetylhexosaminidase contents

Catalog #	Description	Size	M. W.	Purity	pH Range	Storage
GE1101	β-*N*-Acetylhexosaminidase	500 units, lyophilized	51,020	> 95%	3.5-7.5	-20°C, up to 12 months
BA0801	10X Reaction Buffer 4	1 mL			7.5	4 to 25°C
BA1101	10X Reaction Buffer 7	1 mL			5.0	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 β-*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 enzyme 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.

β-*N*-Acetylhexosaminidase is supplied with two 10X Reaction Buffers to ensure optimal digestion and ease of use. Reaction Buffer 4 (Cat #BA0801) is used for reactions that require higher than neutral pH buffering, and Reaction Buffer 7 (Cat #BA1101) is the optimal buffer for most digestions.

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

Activity assay: One unit of enzyme is added to 100 µL of 500 µM pNP-GlcNAc in 1X Reaction Buffer 7 (50 mM sodium citrate, pH 5.0) at 37°C for 30 min, followed by addition of 100 µL of a stop solution (0.2 M sodium borate, pH 9.8). Measure absorption at 405 nm on a plate reader.

Product reconstitution: Dissolve the lyophilized product in 100 µL molecular grade water to make a 5,000 units/mL (Cat #GE1101) solution in 1X Reaction Buffer 4. 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.

Suggested protocol for removal of GlcNAc or GalNAc from oligosaccharides:

1. Mix the following components in a microfuge tube:

Oligosaccharide (e.g., NatGlycan Cat #NG-CM-012)	10 µg
10X Reaction Buffer 7 (Cat #BA1101)	10 µL
β-*N*-Acetylhexosaminidase (Cat #GE1101)	1.0 µL (5 units)
Molecular grade water	to 100 µL final volume

2. Incubate at 37°C for 1 to 4 h.
3. 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

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