

Catalog #	Description	Size	M. W.	Purity	рН	Storage
GE0701	Pan Sialidase	5,000 units, lyophilized	93,476	> 95%	7.0-7.5 optimal	-20°C, up to 12 months
BA0801	10X Reaction Buffer 4	1 mL			7.5	4 to 25°C

## Pan Sialidase contents

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 a recombinant neuraminidase (exo- $\alpha$ -sialidase; glycosyl hydrolase family GH33, EC #3.2.1.18), cloned from *Arthrobacter ureafaciens* and expressed in *Escherichia coli* with an *N*-terminal 8xHis tag. The 8xHis tag may be removed by digestion with FasTEV<sup>TM</sup> (Cat #GE0501), a TEV protease with enhanced stability and catalytic activity.

This product catalyzes the hydrolysis of terminal  $\alpha$ -linked *N*-acetylneuraminic acid (Neu5Ac) from oligosaccharides, complex carbohydrates, and glycoproteins.



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

**Unit definition:** One unit is defined as the amount of Pan Sialidase required to catalyze the release of 1 nanomole of *p*-nitrophenol (pNP) from 2-O-(*p*-nitrophenyl)- $\alpha$ -D-*N*-acetylneuraminic acid (pNP-Neu5Ac) in 1 min at 37°C in 100 µL 1X Reaction Buffer 4 (50 mM Tris-HCl, 100 mM NaCl, pH 7.5).

**Product reconstitution:** Dissolve the lyophilized product in 100 μL of molecular grade water to make a 50,000 units/ml (Cat #GE0701) solution in 1X Reaction Buffer 4. Once reconstituted, store at 4°C for up to 5 days or -20°C for up to 3 months. Aliquoting is recommended to avoid repeated freeze-thaw cycles.

## Suggested protocol for protein desialylation:

1. Mix the following components in a microfuge tube:

Glycoprotein (e.g., fetuin; user supplied) 10X Reaction Buffer 4 (Cat #BA0801) Pan Sialidase (Cat #GE0701) Molecular grade water 1 nanomole (2-100 μg) 10 μL 1.0 μL (50 units) to 100 μL final volume

- 2. Incubate at 37°C for 1 to 4 h.
- Analyze by Western blot to determine the extent of desialylation on the substrate. Suggested 1° probes for Western blot analysis: biotinylated SiaFind<sup>™</sup> α2,3-Specific Lectenz<sup>®</sup> (Cat #SK2301B), SiaFind<sup>™</sup> Pan-Specific Lectenz<sup>®</sup> (Cat #SK0501B) or SiaFind<sup>™</sup> Pan-Specific Lectenz<sup>®</sup> 2.0 (Cat #SK0502B), and SiaFind<sup>™</sup> α2,6-Specific reagent (Cat #SK2601B).

Reference: Christensen S, Egebjerg J. Biotechnol Appl Biochem. 2005 Jun;41(Pt 3):225-31. PMID: 15461582

**Note:** Reactions may be scaled up to accommodate larger amount and volume of substrate. For some substrates, the enzyme may have optimal activity at acidic pH (4.0-6.0). Titration of enzyme amount and pH is recommended