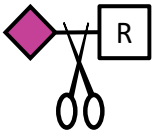
Pan Sialidase contents

Catalog #	Description	Size	M. W.	Purity	pH Range	Storage
GE0701	Pan Sialidase	5,000 units, lyophilized	93,476	> 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 a recombinant neuraminidase (exo-α-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™ (Cat #GE0501), a TEV protease with enhanced stability and catalytic activity.

This enzyme catalyzes the hydrolysis of terminal α-linked *N*-acetylneuraminic acid (Neu5Ac) from oligosaccharides, complex carbohydrates, and glycoproteins.



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

Pan Sialidase 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 Pan Sialidase required to catalyze the release of 1 nanomole of *p*-nitrophenol (pNP) from 2-O-(*p*-nitrophenyl)-α-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).

Activity assay: One unit of enzyme is added to 100 µL of 500 µM pNP-Neu5Ac 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 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)	1 nanomole (2-100 µg)
10X Reaction Buffer 7 (Cat #BA1101)	10 µL
Pan Sialidase (Cat #GE0701)	1.0 µL (50 units)
Molecular grade water	to 100 µL final volume

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

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

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