Rev. 2022-04-13

## α2.3 Sialidase contents

Catalog #	Description	Size	M. W.	Purity	рН	Storage
GE0302-5KU	α2,3 Sialidase	5,000 units, lyophilized	77,512	> 95%	7.0-7.5 optimal	-20°C, up to 12 months
BA0701	10X Reaction Buffer 3	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 a recombinant neuraminidase (exo- $\alpha$ -sialidase, EC #3.2.1.18, CAS #9001-67-6), 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<sup>TM</sup> (Cat #GE0501), a TEV protease with enhanced stability and catalytic activity.

This product preferentially releases terminal  $\alpha 2,3$ -linked *N*-acetylneuraminic acid (Neu5Ac) from oligosaccharides, complex carbohydrates, and glycoproteins.



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

**Unit definition:** One unit is defined as the amount of  $\alpha 2,3$  Sialidase required to catalyze the release of 1 nanomole of p-nitrophenol (pNP) from 2-0-(p-nitrophenyl)- $\alpha$ -D-N-acetylneuraminic acid (pNP-Neu5Ac) in 10 min at 37°C in 100  $\mu$ L 1X Reaction Buffer 3 (50 mM EPPS, 100 mM NaCl, pH 7.5).

**Product reconstitution:** Dissolve the lyophilized product in 100  $\mu$ L of molecular grade water to make a 50,000 units/ml (Cat #GE0302-5KU) solution in 1X Reaction Buffer 3. Once reconstituted, store at 4°C for up to 5 days or -20°C for up to 6 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 3 (Cat #BA0701) α2,3 Sialidase (Cat #GE0302-5KU) Molecular grade water 1 nanomole (2-100 µg)  $10~\mu L$   $1.0~\mu L$  (50 units) to  $100~\mu L$  final volume

- 2. Incubate at 37°C for 1 h.
- 3. Analyze by Western blot or other method 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).

**Note:** Reactions may be scaled up to accommodate larger amount and volume of substrate. Amount of enzyme and reaction time may vary for different substrates. Titration of the amount of enzyme in a reaction is recommended for each new substrate.