

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Certificate of Analysis

| α2-3 Neuraminidase S  |
|---|
| P0743L  |
| 8,000 U/ml  |
| One unit is defined as the amount of enzyme required to cleave > 95% of the terminal $\alpha$ -Neu5Ac from 1 nmol Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-<br>3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 $\mu$ l. |
| 10174019  |
| 12/2024   |
| -20°C   |
| 50 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, (pH 7.5 @ 25°C)  |
| PS-P0743S/L v1.0  |
|   |

| α2-3 Neuraminidase S Component List |                       |            |                      |  |
|-------------------------------------|-----------------------|------------|----------------------|--|
| NEB Part Number                     | Component Description | Lot Number | Individual QC Result |  |
| P0743LVIAL                          | α2-3 Neuraminidase S  | 10174081   | Pass                 |  |
| B1727SVIAL                          | 10X GlycoBuffer 1     | 10153870   | Pass                 |  |

| Assay Name/Specification  | Lot # 10174019 |
|---|----------------|
| <b>Glycosidase Activity (<math>\alpha</math>-N-Acetylgalactosaminidase)</b><br>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled<br>$\alpha$ -N-Acetylgalactosaminidase substrate (GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-AMC) and 80<br>units of $\alpha$ 2-3 Neuraminidase S incubated for 20 hours at 37°C results in no<br>detectable activity as determined by thin layer chromatography. | Pass           |
| <b>Glycosidase Activity (β1-3 Galactosidase)</b><br>A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled<br>β-Galactosidase substrate (Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC) and 80 units of α2-3<br>Neuraminidase S incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography.  | Pass           |
| <b>Glycosidase Activity (<math>\beta</math>-Mannosidase)</b><br>A 10 $\mu$ I reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled<br>$\beta$ -Mannosidase substrate (Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC) and 80 units of $\alpha$ 2-3 Neuraminidase S<br>incubated for 20 hours at 37°C results in no detectable activity as determined by<br>thin layer chromatography.  | Pass           |





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| <b>Glycosidase Activity (<math>\beta</math>-N-Acetylgalactosaminidase)</b><br>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled<br>$\beta$ -N-Acetylgalactosaminidase substrate (GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC) and 80 units of<br>$\alpha$ 2-3 Neuraminidase S incubated for 20 hours at 37°C results in no detectable<br>activity as determined by thin layer chromatography.   | Pass           |
| <b>Glycosidase Activity (<math>\beta</math>1-4 Galactosidase)</b><br>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled<br>$\beta$ -Galactosidase substrate (Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc -AMC) and 80 units of $\alpha$ 2-3<br>Neuraminidase S incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography.        | Pass           |
| <b>Glycosidase Activity (<math>\beta</math>-N-Acetylglucosaminidase)</b><br>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled<br>$\beta$ -N-Acetylglucosaminidase substrate (GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC) and 80 units of<br>$\alpha$ 2-3 Neuraminidase S incubated for 20 hours at 37°C results in no detectable<br>activity as determined by thin layer chromatography. | Pass           |
| <b>Glycosidase Activity (β-Xylosidase)</b><br>A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled<br>β-Xylosidase substrate (Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC) and 80 units of α2-3<br>Neuraminidase S incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography.   | Pass           |
| <b>Glycosidase Activity (Endo F1, F2, H)</b><br>A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F1,<br>F2, H substrate (Dansylated invertase high mannose) and 80 units of α2-3<br>Neuraminidase S incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography.  | Pass           |
| <b>Protein Purity Assay (SDS-PAGE)</b><br>$\alpha$ 2-3 Neuraminidase S is $\geq$ 95% pure as determined by SDS-PAGE analysis using<br>Coomassie Blue detection.  | Pass           |
| Protease Activity (SDS-PAGE)<br>A 20 $\mu$ I reaction in 1X Glyco Buffer 1 containing 24 $\mu$ g of a standard mixture of<br>proteins and a minimum of 80 units of $\alpha$ 2-3 Neuraminidase S incubated for 20 hours<br>at 37°C, results in no detectable degradation of the protein mixture as determined<br>by SDS-PAGE with Coomassie Blue detection.   | Pass           |
| <b>Glycosidase Activity (Endo F2, F3)</b><br>A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F2,<br>F3 substrate (Dansylated fibrinogen biantennary) and 80 units of α2-3 Neuraminidase   | Pass           |





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| S incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.   |                |
| <b>Glycosidase Activity (PNGase F)</b><br>A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled PNGase F<br>substrate (Fluoresceinated fetuin triantennary) and 80 units of α2-3 Neuraminidase S<br>incubated for 20 hours at 37°C results in no detectable activity as determined by<br>thin layer chromatography.  | Pass           |
| <b>Glycosidase Activity (<math>\alpha</math>1-2 Fucosidase)</b><br>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled<br>$\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC) and 80 units of $\alpha$ 2-3 Neuraminidase S<br>incubated for 20 hours at 37°C results in no detectable activity as determined by<br>thin layer chromatography.      | Pass           |
| <b>Glycosidase Activity (α1-3 Fucosidase)</b><br>A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled<br>α-Fucosidase substrate (Fucα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC) and 80 units of α2-3<br>Neuraminidase S incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography.  | Pass           |
| <b>Glycosidase Activity (<math>\alpha</math>1-3 Mannosidase)</b><br>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled<br>$\alpha$ -Mannosidase substrate (Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC) and 80 units of $\alpha$ 2-3<br>Neuraminidase S incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography. | Pass           |
| <b>Glycosidase Activity (α1-3 Galactosidase)</b><br>A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled<br>α-Galactosidase substrate (Galα1-3Galβ1-4GlcNAc-AMC) and 80 units of α2-3<br>Neuraminidase S incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography.  | Pass           |
| <b>Glycosidase Activity (α-Glucosidase)</b><br>A 10 μl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled<br>α-Glucosidase substrate (Glcα1-6Glcα1-4Glc-AMC) and 80 units of α2-3 Neuraminidase S<br>incubated for 20 hours at 37°C results in no detectable activity as determined by<br>thin layer chromatography.  | Pass           |
| <b>Glycosidase Activity (<math>\alpha</math>1-6 Mannosidase)</b><br>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled<br>$\alpha$ -Mannosidase substrate (Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC) and 80 units of $\alpha$ 2-3<br>Neuraminidase S incubated for 20 hours at 37°C results in no detectable activity as                             | Pass           |





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| determined by thin layer chromatography.   |                |
| <b>Glycosidase Activity (<math>\alpha</math>1-6 Galactosidase)</b><br>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled<br>$\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-AMC) and 80 units of $\alpha$ 2-3<br>Neuraminidase S incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography. | Pass           |

This product has been tested and shown to be in compliance with all specifications.

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