

## New England Biolabs Certificate of Analysis

**Product Name:**  $\alpha$ 2-3,6,8 Neuraminidase  
**Catalog Number:** P0720S  
**Concentration:** 50,000 U/ml  
**Unit Definition:** 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 $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-7-amino-4-methyl-coumarin (AMC), in 5 minutes at 37°C in a total reaction volume of 10  $\mu$ l.  
**Packaging Lot Number:** 10062665  
**Expiration Date:** 09/2021  
**Storage Temperature:** -20°C  
**Storage Conditions:** 50 mM NaCl, 20 mM Tris-HCl, 5 mM EDTA, (pH 7.5 @ 25°C)  
**Specification Version:** PS-P0720S/L v1.0

| $\alpha$ 2-3,6,8 Neuraminidase Component List |                                |            |                      |
|---|--------------------------------|------------|----------------------|
| NEB Part Number                               | Component Description          | Lot Number | Individual QC Result |
| P0720SVIAL                                    | $\alpha$ 2-3,6,8 Neuraminidase | 10053879   | Pass                 |
| B1727SVIAL                                    | 10X GlycoBuffer 1              | 10041785   | Pass                 |

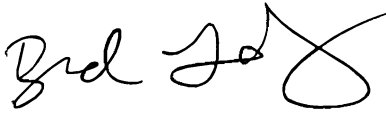
| Assay Name/Specification   | Lot # 10062665 |
|--|----------------|
| <b>Glycosidase Activity (Endo F2, F3)</b><br>A 10 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 500 units of $\alpha$ 2-3,6,8 Neuraminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.       | Pass           |
| <b>Glycosidase Activity (Endo F1, F2, H)</b><br>A 10 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F1, F2, H substrate (Dansylated invertase high mannose) and 500 units of $\alpha$ 2-3,6,8 Neuraminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography. | Pass           |
| <b>Protein Purity Assay (SDS-PAGE)</b><br>$\alpha$ 2-3,6,8 Neuraminidase is $\geq$ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.   | Pass           |
| <b>Glycosidase Activity (<math>\beta</math>-Xylosidase)</b><br>A 10 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled  | Pass           |

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| <p><math>\beta</math>-Xylosidase substrate (Xyl<math>\beta</math>1-4Xyl<math>\beta</math>1-4Xyl<math>\beta</math>1-4Xyl-AMC) and 500 units of <math>\alpha</math>2-3,6,8 Neuraminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>   |                |
| <p><b>Glycosidase Activity (<math>\beta</math>-N-Acetylglucosaminidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\beta</math>-N-Acetylglucosaminidase substrate (GlcNAc<math>\beta</math>1-4GlcNAc<math>\beta</math>1-4GlcNAc-AMC) and 500 units of <math>\alpha</math>2-3,6,8 Neuraminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>           | <b>Pass</b>    |
| <p><b>Glycosidase Activity (<math>\beta</math>-Mannosidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\beta</math>-Mannosidase substrate (Man<math>\beta</math>1-4Man<math>\beta</math>1-4Man-AMC) and 500 units of <math>\alpha</math>2-3,6,8 Neuraminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>  | <b>Pass</b>    |
| <p><b>Glycosidase Activity (<math>\beta</math>-N-Acetylgalactosaminidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\beta</math>-N-Acetylgalactosaminidase substrate (GalNAc<math>\beta</math>1-4Gal<math>\beta</math>1-4Glc-AMC) and 500 units of <math>\alpha</math>2-3,6,8 Neuraminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>             | <b>Pass</b>    |
| <p><b>Glycosidase Activity (<math>\beta</math>1-4 Galactosidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\beta</math>-Galactosidase substrate (Gal<math>\beta</math>1-4GlcNAc<math>\beta</math>1-3Gal<math>\beta</math>1-4Glc -AMC) and 500 units of <math>\alpha</math>2-3,6,8 Neuraminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>         | <b>Pass</b>    |
| <p><b>Glycosidase Activity (<math>\alpha</math>-N-Acetylgalactosaminidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\alpha</math>-N-Acetylgalactosaminidase substrate (GalNAc<math>\alpha</math>1-3(Fuca1-2)Gal<math>\beta</math>1-4Glc-AMC) and 500 units of <math>\alpha</math>2-3,6,8 Neuraminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p> | <b>Pass</b>    |
| <p><b>Glycosidase Activity (<math>\beta</math>1-3 Galactosidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\beta</math>-Galactosidase substrate (Gal<math>\beta</math>1-3GlcNAc<math>\beta</math>1-4Gal<math>\beta</math>1-4Glc-AMC) and 500 units of <math>\alpha</math>2-3,6,8 Neuraminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>          | <b>Pass</b>    |
| <p><b>Glycosidase Activity (<math>\alpha</math>-Glucosidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Glucosidase substrate (Glc<math>\alpha</math>1-6Glc<math>\alpha</math>1-4Glc-AMC) and 500 units of <math>\alpha</math>2-3,6,8</p>   | <b>Pass</b>    |

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| <p>Neuraminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>   |                |
| <p><b>Glycosidase Activity (α1-6 Mannosidase)</b><br/>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-6Manα1-6(Manα1-3)Man-AMC) and 500 units of α2-3,6,8 Neuraminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>       | <b>Pass</b>    |
| <p><b>Glycosidase Activity (α1-6 Galactosidase)</b><br/>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-6Galα1-6Glcα1-2Fru-AMC) and 500 units of α2-3,6,8 Neuraminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>     | <b>Pass</b>    |
| <p><b>Glycosidase Activity (α1-3 Fucosidase)</b><br/>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fuca1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC) and 500 units of α2-3,6,8 Neuraminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p> | <b>Pass</b>    |
| <p><b>Glycosidase Activity (α1-3 Galactosidase)</b><br/>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-3Galβ1-4GlcNAc-AMC) and 500 units of α2-3,6,8 Neuraminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>         | <b>Pass</b>    |
| <p><b>Glycosidase Activity (α1-3 Mannosidase)</b><br/>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-3Manβ1-4GlcNAc-AMC) and 500 units of α2-3,6,8 Neuraminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>             | <b>Pass</b>    |
| <p><b>Glycosidase Activity (α1-2 Fucosidase)</b><br/>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fuca1-2Galβ1-4Glc-AMC) and 500 units of α2-3,6,8 Neuraminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>                  | <b>Pass</b>    |
| <p><b>Protease Activity (SDS-PAGE)</b><br/>A 20 µl reaction in 1X Glyco Buffer 1 containing 24 µg of a standard mixture of proteins and a minimum of 500 units of α2-3,6,8 Neuraminidase incubated for 20 hours at 37°C, results in no detectable degradation of the protein mixture as</p>   | <b>Pass</b>    |

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|---|--------------------|
| <p>determined by SDS-PAGE with Coomassie Blue detection.</p> <p><b>Glycosidase Activity (PNGase F)</b><br/>A 10 µl reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled PNGase F substrate (Fluoresceinated fetuin triantennary) and 500 units of α-2-3,6,8 Neuraminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p> | <p><b>Pass</b></p> |

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



Brad Landgraf  
Production Scientist  
12 Sep 2019



Michael Tonello  
Packaging Quality Control Inspector  
31 Jan 2020