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New England Biolabs Certificate of Analysis

Product Name: $\alpha 2-3,6,8$ Neuraminidase

Catalog #: P0720S/L
Concentration: 50,000 units/ml

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α -Neu5Ac from 1 nmol Neu5Ac α 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

 $10 \mu l$.

 Lot #:
 0161603

 Assay Date:
 03/2016

 Expiration Date:
 03/2018

 Storage Temp:
 -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
Effective Date: 19 Feb 2016

Assay Name/Specification (minimum release criteria)	Lot #0161603
Glycosidase Activity (Endo F1, F2, H) - A 10 μ 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 α 2-3,6,8 Neuraminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (Endo F2, F3) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) 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.	Pass
Glycosidase Activity (PNGase F) - A 10 μ l 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.	Pass
Glycosidase Activity (β-Mannosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-Mannosidase substrate (Manβ1-4Manβ1-4Man-AMC) 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.	Pass









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Assay Name/Specification (minimum release criteria)	Lot #0161603
Glycosidase Activity (β -N-Acetylgalactosaminidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β -N-Acetylgalactosaminidase substrate (GalNAc β 1-4Gal β 1-4Glc-AMC) 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.	Pass
Glycosidase Activity (β -N-Acetylglucosaminidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β -N-Acetylglucosaminidase substrate (GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC) 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.	Pass
Glycosidase Activity (β -Xylosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β -Xylosidase substrate (Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-AMC) 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.	Pass
Glycosidase Activity (β 1-3 Galactosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β -Galactosidase substrate (Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC) 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.	Pass
Glycosidase Activity (β 1-4 Galactosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β -Galactosidase substrate ($Gal\beta$ 1-4 $GlcNAc\beta$ 1-3 $Gal\beta$ 1-4 Glc -AMC) 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.	Pass
Glycosidase Activity (α -Glucosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -Glucosidase substrate (Glc α 1-6Glc α 1-4Glc-AMC) 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.	Pass
Glycosidase Activity (α -N-Acetylgalactosaminidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -N-Acetylgalactosaminidase substrate (GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc-AMC) 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.	Pass
Glycosidase Activity (α 1-2 Fucosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -Fucosidase substrate (Fuc α 1-2Gal β 1-4Glc-AMC) 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.	Pass









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Glycosidase Activity (α 1-3 Fucosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -Fucosidase substrate (Fuc α 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc-AMC) 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.	Pass
Glycosidase Activity (α 1-3 Galactosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -Galactosidase substrate (Gal α 1-3Gal β 1-4GlcNAc-AMC) 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.	Pass
Glycosidase Activity ($\alpha 1$ -3 Mannosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -Mannosidase substrate (Man $\alpha 1$ -3Man $\beta 1$ -4GlcNAc-AMC) 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
Glycosidase Activity (α 1-6 Galactosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -Galactosidase substrate (Gal α 1-6Gal α 1-6Glc α 1-2Fru-AMC) 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.	Pass
Glycosidase Activity (α 1-6 Mannosidase) - A 10 μ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α -Mannosidase substrate (Man α 1-6Man α 1-6(Man α 1-3)Man-AMC) 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.	Pass
Protease Activity (SDS-PAGE) - A 20 μ l reaction in 1X Glyco Buffer 1 containing 24 μ g of a standard mixture of proteins and a minimum of 500 units of α 2-3,6,8 Neuraminidase incubated for 20 hours at 37°C, results in no detectable degradation of the protein mixture as determined by SDS-PAGE with Coomassie Blue detection.	Pass
Protein Purity Assay (SDS-PAGE) - $\alpha 2$ -3,6,8 Neuraminidase is \geq 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass

Authorized by Derek Robinson 19 Feb 2016







Inspected by Alicia Bielik 04 Mar 2016