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

Product Name:  $\alpha$ -N-Acetylgalactosaminidase

Catalog #: P0734S/L
Concentration: 20,000 units/ml

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal  $\alpha$ -D-N-acetylgalactosamine from 1 nmol

 $(GalNAc\alpha 1-3)(Fuc\alpha 1-2)Gal\beta 1-4Glc-7-amino-4-methyl-coumarin (AMC)$ , in 1 hour at 37°C in a total reaction volume of

10 μl.

 Lot #:
 0071610

 Assay Date:
 10/2016

 Expiration Date:
 10/2018

 Storage Temp:
 -20°C

Storage Conditions: 50 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, (pH 7.5 @ 25°C)

Specification Version: PS-P0734S/L v1.0 Effective Date: 17 Feb 2016

Assay Name/Specification (minimum release criteria)	Lot #0071610
Glycosidase Activity (Endo F1, F2, H) - 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 20 units of $\alpha$ -N-Acetylgalactosaminidase 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 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 20 units of $\alpha$ -N-Acetylgalactosaminidase 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 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled PNGase F substrate (Fluoresceinated fetuin triantennary) and 20 units of $\alpha$ -N-Acetylgalactosaminidase 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 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-Mannosidase substrate (Manβ1-4Manβ1-4Man-AMC) and 20 units of $\alpha$ -N-Acetylgalactosaminidase 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 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled β-Xylosidase substrate (Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC) and 20 units of $\alpha$ -N-Acetylgalactosaminidase 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 #0071610
Glycosidase Activity ( $\beta$ 1-3 Galactosidase) - A 10 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled $\beta$ -Galactosidase substrate (Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC) and 20 units of $\alpha$ -N-Acetylgalactosaminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity ( $\beta$ 1-4 Galactosidase) - A 10 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled $\beta$ -Galactosidase substrate (Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc -AMC) and 20 units of $\alpha$ -N-Acetylgalactosaminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity ( $\beta$ -N-Acetylgalactosaminidase) - A 10 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled $\beta$ -N-Acetylgalactosaminidase substrate (GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC) and 20 units of $\alpha$ -N-Acetylgalactosaminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity ( $\beta$ -N-Acetylglucosaminidase) - A 10 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled $\beta$ -N-Acetylglucosaminidase substrate (GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC) and 20 units of $\alpha$ -N-Acetylgalactosaminidase 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 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled α-Glucosidase substrate (Glcα1-6Glcα1-4Glc-AMC) and 20 units of α-N-Acetylgalactosaminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity ( $\alpha$ -Neuraminidase) - A 10 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled $\alpha$ -Neuraminidase substrate (Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 20 units of $\alpha$ -N-Acetylgalactosaminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity ( $\alpha$ 1-2 Fucosidase) - A 10 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC) and 20 units of $\alpha$ - $N$ -Acetylgalactosaminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity ( $\alpha 1$ -3 Fucosidase) - A 10 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled $\alpha$ -Fucosidase substrate (Fuc $\alpha 1$ -3Gal $\beta 1$ -4GlcNAc $\beta 1$ -3Gal $\beta 1$ -4Glc-AMC) and 20 units of $\alpha$ -N-Acetylgalactosaminidase 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 #0071610
Glycosidase Activity ( $\alpha 1$ -3 Galactosidase) - A 10 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled $\alpha$ -Galactosidase substrate (Gal $\alpha 1$ -3Gal $\beta 1$ -4GlcNAc-AMC) and 20 units of $\alpha$ -N-Acetylgalactosaminidase 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 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled $\alpha$ -Mannosidase substrate (Man $\alpha 1$ -3Man $\beta 1$ -4GlcNAc-AMC) and 20 units of $\alpha$ -N-Acetylgalactosaminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity ( $\alpha 1$ -6 Galactosidase) - A 10 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled $\alpha$ -Galactosidase substrate (Gal $\alpha 1$ -6Gal $\alpha 1$ -6Glc $\alpha 1$ -2Fru-AMC) and 20 units of $\alpha$ -N-Acetylgalactosaminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity ( $\alpha$ 1-6 Mannosidase) - A 10 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled $\alpha$ -Mannosidase substrate (Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC) and 20 units of $\alpha$ -N-Acetylgalactosaminidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Protease Activity (SDS-PAGE)</b> - A 20 $\mu$ l reaction in 1X Glyco Buffer 1 containing 24 $\mu$ g of a standard mixture of proteins and a minimum of 100 units of $\alpha$ - $N$ -Acetylgalactosaminidase 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
<b>Protein Purity Assay (SDS-PAGE)</b> - $\alpha$ - $N$ -Acetylgalactosaminidase is $\geq$ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass

Authorized by Derek Robinson 17 Feb 2016







Inspected by Alicia Bielik 21 Oct 2016