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: Endo Hf
Catalog #: P0703S/L

Concentration: 1,000,000 units/ml

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10  $\mu$ g of denatured RNase B in

1 hour at 37°C in a total reaction volume of 10  $\mu$ l (10 NEB units = 1 IUB milliunit).

 Lot #:
 0181703

 Assay Date:
 03/2017

 Expiration Date:
 03/2019

 Storage Temp:
 -20°C

Storage Conditions: 50 mM NaCl, 20 mM Tris-HCl, 5 mM EDTA, (pH 7.5 @ 25°C)

Specification Version: PS-P0703S/L v1.0
Effective Date: 12 Feb 2016

Assay Name/Specification (minimum release criteria)	Lot #0181703
Glycosidase Activity (Endo F2, F3) - A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 5,000 units of Endo Hf 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 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled PNGase F substrate (Fluoresceinated fetuin triantennary) and 5,000 units of Endo Hf 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 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled β-Mannosidase substrate (Manβ1-4Manβ1-4Man-AMC) and 5,000 units of Endo Hf 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 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled β-Xylosidase substrate (Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC) and 5,000 units of Endo Hf incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (β1-3 Galactosidase) - A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC) and 5,000 units of Endo Hf incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (β1-4 Galactosidase) - A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-4GlcNAcβ1-3Galβ1-4Glc -AMC) and 5,000 units of Endo Hf 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 ( $\beta$ -N-Acetylgalactosaminidase) - A 10 $\mu$ l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled $\beta$ -N-Acetylgalactosaminidase substrate (GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC) and 5,000 units of Endo Hf 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 3 containing 1 nM of fluorescently-labeled $\beta$ -N-Acetylglucosaminidase substrate (GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC) and 5,000 units of Endo Hf incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity ( $\alpha$ -Glucosidase) - A 10 $\mu$ l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled $\alpha$ -Glucosidase substrate (Glc $\alpha$ 1-4Glc-AMC) and 5,000 units of Endo Hf incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α-Neuraminidase) - A 10 $\mu$ l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled α-Neuraminidase substrate (Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC) and 5,000 units of Endo Hf 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 3 containing 1 nM of fluorescently-labeled $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC) and 5,000 units of Endo Hf 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 3 containing 1 nM of fluorescently-labeled $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 5,000 units of Endo Hf incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity ( $\alpha$ 1-3 Galactosidase) - A 10 $\mu$ l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc-AMC) and 5,000 units of Endo Hf 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 3 containing 1 nM of fluorescently-labeled $\alpha$ -Mannosidase substrate (Man $\alpha 1$ -3Man $\beta 1$ -4GlcNAc-AMC) and 5,000 units of Endo Hf 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 3 containing 1 nM of fluorescently-labeled $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-AMC) and 5,000 units of Endo Hf 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 3 containing 1 nM of fluorescently-labeled $\alpha$ -Mannosidase substrate (Man $\alpha 1$ -6Man $\alpha 1$ -6(Man $\alpha 1$ -3)Man-AMC) and 5,000 units of Endo Hf 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 ( $\alpha$ -N-Acetylgalactosaminidase) - A 10 $\mu$ l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled $\alpha$ -N-Acetylgalactosaminidase substrate (GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-AMC) and 5,000 units of Endo Hf 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 μl reaction in 1X Glyco Buffer 3 containing 24 μg of a standard mixture of proteins and a minimum of 5,000 units of Endo Hf 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> - Endo Hf is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass

Authorized by Derek Robinson 12 Feb 2016







Inspected by Alicia Bielik 23 Mar 2017