

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 H
Catalog Number:	P0702S
Concentration:	500,000 U/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).
Packaging Lot Number:	10124277
Expiration Date:	09/2023
Storage Temperature:	-20°C
Storage Conditions:	50 mM NaCl , 20 mM Tris-HCl , 5 mM EDTA, (pH 7.5 @ 25°C)
Specification Version:	PS-P0702S/L v1.0

Endo H Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
P0702SVIAL	Endo H	10115332	Pass	
B1720SVIAL	10X Glycobuffer 3	10120516	Pass	
B1704SVIAL	Glycoprotein Denaturing Buffer	10119058	Pass	

Assay Name/Specification	Lot # 10124277
<b>Glycosidase Activity (<math>\beta</math>1-4 Galactosidase)</b> A 10 µl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled $\beta$ -Galactosidase substrate (Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc -AMC) and 5,000 units of Endo H incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (β1-3 Galactosidase)</b> 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 H incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\alpha</math>-Neuraminidase)</b> A 10 µl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled $\alpha$ -Neuraminidase substrate (Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 5,000 units of Endo H incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass





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<b>Glycosidase Activity (<math>\alpha</math>-N-Acetylgalactosaminidase)</b> A 10 $\mu$ I 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 H incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\alpha</math>-Glucosidase)</b> A 10 µl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled $\alpha$ -Glucosidase substrate (Glc $\alpha$ 1-6Glc $\alpha$ 1-4Glc-AMC) and 5,000 units of Endo H incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (α1-6 Mannosidase)</b> A 10 µl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-6Manα1-6(Manα1-3)Man-AMC) and 5,000 units of Endo H incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\beta</math>-N-Acetylglucosaminidase)</b> A 10 µl 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 H incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\beta</math>-N-Acetylgalactosaminidase)</b> A 10 µl 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 H incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\beta</math>-Mannosidase)</b> A 10 $\mu$ I reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled $\beta$ -Mannosidase substrate (Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC) and 5,000 units of Endo H incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (β-Xylosidase)</b> 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 H incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass





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<b>Glycosidase Activity (<math>\alpha</math>1-6 Galactosidase)</b> A 10 µl 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 H incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\alpha</math>1-3 Mannosidase)</b> A 10 µl 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 H incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (α1-3 Galactosidase)</b> A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-3Galβ1-4GlcNAc-AMC) and 5,000 units of Endo H incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (α1-3 Fucosidase)</b> A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC) and 5,000 units of Endo H incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (α1-2 Fucosidase)</b> A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-2Galβ1-4Glc-AMC) and 5,000 units of Endo H 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 H 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) Endo H is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
<b>Glycosidase Activity (PNGase F)</b> 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 H incubated	Pass





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for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	
<b>Glycosidase Activity (Endo F2, F3)</b> 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 H incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass

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

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Alicia Bielik Production Scientist 19 Nov 2021

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Mary Neal Packaging Quality Control Inspector 19 Nov 2021

