

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:	10077476
Expiration Date:	07/2022
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	10077475	Pass	
B1720SVIAL	10X Glyco Buffer 3	10048943	Pass	
B1704SVIAL	Glycoprotein Denaturing Buffer	10065640	Pass	

Assay Name/Specification	Lot # 10077476
<b>Glycosidase Activity (β-Mannosidase)</b> 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 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>1-6 Mannosidase)</b> A 10 µl 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 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>1-6 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





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<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 (<math>\alpha</math>1-3 Galactosidase)</b> A 10 µl 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 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 Fucosidase)</b> A 10 µl 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 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>1-2 Fucosidase)</b> A 10 µl 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 H incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (α-Neuraminidase)</b> A 10 µl 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 H incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (α-N-Acetylgalactosaminidase)</b> A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled α-N-Acetylgalactosaminidase substrate (GalNAcα1-3(Fucα1-2)Galβ1-4Glc-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>Protein Purity Assay (SDS-PAGE)</b> Endo H is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
Protease Activity (SDS-PAGE)	Pass





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A 20 $\mu$ I reaction in 1X Glyco Buffer 3 containing 24 $\mu$ g 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.	
<b>Glycosidase Activity (β1-4 Galactosidase)</b> 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 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>\beta</math>-Xylosidase)</b> A 10 $\mu$ I reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled $\beta$ -Xylosidase substrate (Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl-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
<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>\alpha</math>-Glucosidase)</b> A 10 $\mu$ l 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 (PNGase F)</b> A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled PNGase F	Pass





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substrate (Fluoresceinated fetuin triantennary) 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.	
<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 28 Jul 2020

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