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## New England Biolabs Certificate of Analysis

Product Name: Endo Hf
Catalog Number: P0703L

Concentration: 1,000,000 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: 10102423
Expiration Date: 02/2023
Storage Temperature: -20°C

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

Specification Version: PS-P0703S/L v1.0

Endo Hf Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
P0703LVIAL	Endo Hf	10097695	Pass	
B1720SVIAL	10X Glycobuffer 3	10082734	Pass	
B1704SVIAL	Glycoprotein Denaturing Buffer	10090539	Pass	

Assay Name/Specification	Lot # 10102423
Protein Purity Assay (SDS-PAGE) Endo Hf is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue	Pass
detection.	
Protease Activity (SDS-PAGE) A 20 µl reaction in 1X Glyco Buffer 3 containing 24 µg of a standard mixture of	Pass
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.	
Glycosidase Activity (β1-4 Galactosidase)	Pass
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.	
Glycosidase Activity (β1-3 Galactosidase)	Pass



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Assay Name/Specification	Lot # 10102423
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.	
Glycosidase Activity (β-N-Acetylgalactosaminidase) A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled β-N-Acetylgalactosaminidase substrate (GalNAcβ1-4Galβ1-4Glc-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 (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 (α-Glucosidase) A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled α-Glucosidase substrate (Glcα1-6Glcα1-4Glc-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 (α1-6 Mannosidase) A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Μαnα1-6Μαnα1-6(Μαnα1-3)Μαn-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 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 Hf 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 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 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	Pass



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Assay Name/Specification	Lot # 10102423
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.	
Glycosidase Activity (β-N-Acetylglucosaminidase) A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled β-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
<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 Hf incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α1-3 Fucosidase) 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 Hf incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α1-6 Galactosidase) A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-6Galα1-6Glcα1-2Fru-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 (α1-3 Mannosidase) A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-3Manβ1-4GlcNAc-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 (α1-2 Fucosidase) 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 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	Pass



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

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Alicia Bielik Production Scientist 16 Mar 2021

thin layer chromatography.

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Packaging Quality Control Inspector

16 Mar 2021

