NEW ENGLAND BioLabs Inc.
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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 µg of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl (10 NEB units = 1 IUB milliunit).##*
Packaging Lot Number:	*10145052*
Expiration Date:	*04/2024*
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

NEB Part Number	Component Description	Lot Number	Individual QC Result
P0703LVIAL	Endo Hf	10145051	**Pass**
B1720SVIAL	10X Glycobuffer 3	10120516	**Pass**
B1704SVIAL	Glycoprotein Denaturing Buffer	10119058	**Pass**

Assay Name/Specification	Lot # 10145052
Glycosidase Activity (α1-6 Mannosidase) 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 Hf incubated for 20 hours at 37ºC 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 37ºC 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 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**

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Assay Name/Specification	Lot # 10145052
Glycosidase Activity (α1-3 Galactosidase) 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 37ºC 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 37ºC 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 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**
Protein Purity Assay (SDS-PAGE) Endo Hf is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**
Protease Activity (SDS-PAGE) 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**
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 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**
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 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**
Glycosidase Activity (α-Glucosidase)	**Pass**

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Assay Name/Specification	Lot # 10145052
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 37ºC results in no detectable activity as determined by thin layer chromatography.	
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 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**
Glycosidase Activity (β-N-Acetylglucosaminidase) A 10 µl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled β-N-Acetylglucosaminidase substrate (GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC) and 5,000 units of Endo Hf incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**
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 37ºC 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 37ºC 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 37ºC 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 37ºC 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	**Pass**

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Assay Name/Specification	Lot # 10145052
α-Neuraminidase substrate (Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC) and 5,000 units of Endo Hf incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography.	
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 37ºC 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.

Alicia Bielik
Production Scientist
05 May 2022

Erin Varney
Packaging Quality Control Inspector
05 May 2022