

## 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 µg of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl (10 NEB units = 1 IUB milliumit).  
**Lot #:** 0181711  
**Assay Date:** 11/2017  
**Expiration Date:** 11/2019  
**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 #0181711
<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 Hf incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<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 Hf incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<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 Hf incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<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 Hf incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<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 Hf incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<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 Hf incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>



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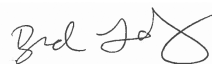
Assay Name/Specification (minimum release criteria)	Lot #0181711
<b>Glycosidase Activity (<math>\beta</math>-N-Acetylgalactosaminidase)</b> - A 10 $\mu$ l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled $\beta$ -N-Acetylgalactosaminidase substrate (GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC) 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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\beta</math>-N-Acetylglucosaminidase)</b> - 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.	<b>Pass</b>
<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 Hf incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>-Neuraminidase)</b> - A 10 $\mu$ l 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 Hf incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-2 Fucosidase)</b> - 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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-3 Fucosidase)</b> - 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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-3 Galactosidase)</b> - 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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-3 Mannosidase)</b> - 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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-6 Galactosidase)</b> - 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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-6 Mannosidase)</b> - 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.	<b>Pass</b>

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<p><b>Glycosidase Activity (<math>\alpha</math>-N-Acetylgalactosaminidase)</b> - A 10 <math>\mu</math>l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled <math>\alpha</math>-N-Acetylgalactosaminidase substrate (GalNAc<math>\alpha</math>1-3(Fuc<math>\alpha</math>1-2)Gal<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Protease Activity (SDS-PAGE)</b> - A 20 <math>\mu</math>l reaction in 1X Glyco Buffer 3 containing 24 <math>\mu</math>g 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.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> - Endo Hf is <math>\geq</math> 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>



Authorized by  
Derek Robinson  
12 Feb 2016



Inspected by  
Brad Landgraf  
06 Dec 2017

