

## New England Biolabs Certificate of Analysis

**Product Name:** Endo Hf  
**Catalog Number:** P0703S  
**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:** 10152109  
**Expiration Date:** 05/2024  
**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 |
| P0703SVIAL             | Endo Hf                        | 10152108   | Pass                 |
| B1720SVIAL             | 10X Glycobuffer 3              | 10120516   | Pass                 |
| B1704SVIAL             | Glycoprotein Denaturing Buffer | 10119058   | Pass                 |

| Assay Name/Specification                                                                                                                                                                                                                                                                                                                                               | Lot # 10152109 |
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| <p><b>Glycosidase Activity (β-Xylosidase)</b><br/>           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.</p>                             | Pass           |
| <p><b>Glycosidase Activity (β-N-Acetylglucosaminidase)</b><br/>           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 37°C results in no detectable activity as determined by thin layer chromatography.</p> | Pass           |
| <p><b>Glycosidase Activity (β-N-Acetylgalactosaminidase)</b><br/>           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.</p>   | Pass           |

| Assay Name/Specification                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Lot # 10152109 |
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| <p><b>Glycosidase Activity (<math>\beta</math>-Mannosidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled <math>\beta</math>-Mannosidase substrate (Man<math>\beta</math>1-4Man<math>\beta</math>1-4Man-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.</p>                                                              | <b>Pass</b>    |
| <p><b>Glycosidase Activity (<math>\beta</math>1-4 Galactosidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled <math>\beta</math>-Galactosidase substrate (Gal<math>\beta</math>1-4GlcNAc<math>\beta</math>1-3Gal<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>Glycosidase Activity (<math>\beta</math>1-3 Galactosidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled <math>\beta</math>-Galactosidase substrate (Gal<math>\beta</math>1-3GlcNAc<math>\beta</math>1-4Gal<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>Glycosidase Activity (<math>\alpha</math>-Neuraminidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Neuraminidase substrate (Neu5Ac<math>\alpha</math>2-3Gal<math>\beta</math>1-3GlcNAc<math>\beta</math>1-3Gal<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>Glycosidase Activity (<math>\alpha</math>-N-Acetylgalactosaminidase)</b><br/>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(Fuca1-2)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>Glycosidase Activity (<math>\alpha</math>-Glucosidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Glucosidase substrate (Glc<math>\alpha</math>1-6Glc<math>\alpha</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>Glycosidase Activity (<math>\alpha</math>1-6 Mannosidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Mannosidase substrate (Man<math>\alpha</math>1-6Man<math>\alpha</math>1-6(Man<math>\alpha</math>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.</p>                            | <b>Pass</b>    |

| Assay Name/Specification                                                                                                                                                                                                                                                                                                                     | Lot # 10152109 |
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| <p><b>Glycosidase Activity (α1-6 Galactosidase)</b><br/>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.</p>     | <b>Pass</b>    |
| <p><b>Glycosidase Activity (α1-3 Mannosidase)</b><br/>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.</p>             | <b>Pass</b>    |
| <p><b>Glycosidase Activity (α1-3 Galactosidase)</b><br/>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.</p>         | <b>Pass</b>    |
| <p><b>Glycosidase Activity (α1-3 Fucosidase)</b><br/>A 10 µl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fuca1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-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.</p> | <b>Pass</b>    |
| <p><b>Glycosidase Activity (α1-2 Fucosidase)</b><br/>A 10 µl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fuca1-2Galβ1-4Glc-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.</p>                  | <b>Pass</b>    |
| <p><b>Protein Purity Assay (SDS-PAGE)</b><br/>Endo Hf is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>                                                                                                                                                                                                   | <b>Pass</b>    |
| <p><b>Protease Activity (SDS-PAGE)</b><br/>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.</p>         | <b>Pass</b>    |
| <p><b>Glycosidase Activity (PNGase F)</b><br/>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</p>                                                                                                                  | <b>Pass</b>    |

| Assay Name/Specification                                                                                                                                                                                                                                                                                                                                                                                                                     | Lot # 10152109     |
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| <p>for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p> <p><b>Glycosidase Activity (Endo F2, F3)</b><br/>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.</p> | <p><b>Pass</b></p> |

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

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19 May 2022



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