

## New England Biolabs Certificate of Analysis

**Product Name:** Remove-iT<sup>®</sup> PNGase F  
**Catalog Number:** P0706L  
**Concentration:** 225,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 5 µg of DTT denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl.  
**Packaging Lot Number:** 10102195  
**Expiration Date:** 03/2022  
**Storage Temperature:** 4°C  
**Storage Conditions:** 50 mM NaCl , 20 mM Tris-HCl , 5 mM EDTA, (pH 7.5 @ 25°C)  
**Specification Version:** PS-P0706S/L v1.0

Remove-iT <sup>®</sup> PNGase F Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
P0706LVIAL	Remove-iT <sup>®</sup> PNGase F	10102194	Pass
B3704SVIAL	10X GlycoBuffer 2	10091370	Pass
B0706SVIAL	10X DTT	10084215	Pass

Assay Name/Specification	Lot # 10102195
<p><b>Glycosidase Activity (β-Xylosidase)</b>            A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-Xylosidase substrate (Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC) and 450 units of Remove-iT<sup>®</sup> PNGase F 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>            A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-N-Acetylglucosaminidase substrate (GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC) and 450 units of Remove-iT<sup>®</sup> PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (β1-4 Galactosidase)</b>            A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-4GlcNAcβ1-3Galβ1-4Glc -AMC) and 450 units of Remove-iT<sup>®</sup> PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass

Assay Name/Specification	Lot # 10102195
<p><b>Glycosidase Activity (<math>\beta</math>-N-Acetylgalactosaminidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-N-Acetylgalactosaminidase substrate (GalNAc<math>\beta</math>1-4Gal<math>\beta</math>1-4Glc-AMC) and 450 units of Remove-iT<sup>®</sup> PNGase F 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 2 containing 24 <math>\mu</math>g of a standard mixture of proteins and a minimum of 1,125 units of Remove-iT<sup>®</sup> PNGase F 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 (<math>\beta</math>-Mannosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-Mannosidase substrate (Man<math>\beta</math>1-4Man<math>\beta</math>1-4Man-AMC) and 450 units of Remove-iT<sup>®</sup> PNGase F 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>1-3 Fucosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Fucosidase substrate (Fuc<math>\alpha</math>1-3Gal<math>\beta</math>1-4GlcNAc<math>\beta</math>1-3Gal<math>\beta</math>1-4Glc-AMC) and 450 units of Remove-iT<sup>®</sup> PNGase F 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>1-3 Mannosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Mannosidase substrate (Man<math>\alpha</math>1-3Man<math>\beta</math>1-4GlcNAc-AMC) and 450 units of Remove-iT<sup>®</sup> PNGase F 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>1-3 Galactosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Galactosidase substrate (Gal<math>\alpha</math>1-3Gal<math>\beta</math>1-4GlcNAc-AMC) and 450 units of Remove-iT<sup>®</sup> PNGase F 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>1-2 Fucosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Fucosidase substrate (Fuc<math>\alpha</math>1-2Gal<math>\beta</math>1-4Glc-AMC) and 450 units of Remove-iT<sup>®</sup> PNGase F 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 # 10102195
<p><b>Protein Purity Assay (SDS-PAGE)</b> Remove-iT<sup>®</sup> PNGase F is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass
<p><b>Glycosidase Activity (β1-3 Galactosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC) and 450 units of Remove-iT<sup>®</sup> PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (α1-6 Mannosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-6Manα1-6(Manα1-3)Man-AMC) and 450 units of Remove-iT<sup>®</sup> PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (α1-6 Galactosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-6Galα1-6Glcα1-2Fru-AMC) and 450 units of Remove-iT<sup>®</sup> PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (α-N-Acetylgalactosaminidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-N-Acetylgalactosaminidase substrate (GalNAcα1-3(Fuca1-2)Galβ1-4Glc-AMC) and 450 units of Remove-iT<sup>®</sup> PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (α-Neuraminidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Neuraminidase substrate (Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC) and 450 units of Remove-iT<sup>®</sup> PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (α-Glucosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Glucosidase substrate (Glcα1-6Glcα1-4Glc-AMC) and 450 units of Remove-iT<sup>®</sup> PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Endoglycosidase F1 Activity</b> A 20 µl reaction in Glyco Buffer 2 containing 20 pmol of fluorescently-labeled 2-AA</p>	Pass

Assay Name/Specification	Lot # 10102195
<p>Man-5 fluorescent standard and 1,125 units of Remove-iT® PNGase F incubated for 20 hours at 37°C results in no endoglycosidase F1 activity as determined by LC/MS analysis with fluorescent detection.</p>	
<p><b>Glycosidase Activity (Endo F2, F3)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 450 units of Remove-iT® PNGase F 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 (Endo F1, F2, H)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled Endo F1, F2, H substrate (Dansylated invertase high mannose) and 450 units of Remove-iT® PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<b>Pass</b>
<p><b>Functional Test (Magnetic Beads, Enzyme Removal)</b> Magnetic chitin beads ( 50 µl ) were equilibrated and incubated with 1,125 units of Remove-iT® PNGase F in 300 µl of 50 mM ammonium formate, pH 4.4 . The beads were pelleted using a magnetic separation rack. No Remove-iT® PNGase F was detected in the supernatant as determined by activity assay and mass spectrometry analysis.</p>	<b>Pass</b>

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit [www.neb.com/trademarks](http://www.neb.com/trademarks) for additional information.



Alicia Bielik  
Production Scientist  
22 Apr 2021



Josh Hersey  
Packaging Quality Control Inspector  
22 Apr 2021