

## New England Biolabs Certificate of Analysis

*Product Name:* Remove-iT<sup>®</sup> PNGase F  
*Catalog #:* P0706S/L  
*Concentration:* 225,000 units/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.  
*Lot #:* 0031805  
*Assay Date:* 05/2018  
*Expiration Date:* 05/2019  
*Storage Temp:* 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  
*Effective Date:* 26 Feb 2016

Assay Name/Specification (minimum release criteria)	Lot #0031805
<b>Endoglycosidase F1 Activity</b> - A 20 µl reaction in Glyco Buffer 2 containing 20 pmol of fluorescently-labeled 2-AA Man-5 fluorescent standard and 1,125 units of Remove-iT <sup>®</sup> PNGase F incubated for 20 hours at 37°C results in no endoglycosidase F1 activity as determined by LC/MS analysis with fluorescent detection.	<b>Pass</b>
<b>Functional Test (Magnetic Beads, Enzyme Removal)</b> - Magnetic chitin beads ( 50 µl ) were equilibrated and incubated with 1,125 units of Remove-iT <sup>®</sup> 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 <sup>®</sup> PNGase F was detected in the supernatant as determined by activity assay and mass spectrometry analysis.	<b>Pass</b>
<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 <sup>®</sup> PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<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 <sup>®</sup> PNGase F 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 2 containing 1 nM of fluorescently-labeled β-Mannosidase substrate (Manβ1-4Manβ1-4Man-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.	<b>Pass</b>



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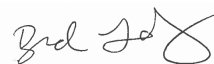
Assay Name/Specification (minimum release criteria)	Lot #0031805
<b>Glycosidase Activity (β-Xylosidase)</b> - A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-Xylosidase substrate (Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC) and 450 units of Remove-iT <sup>®</sup> PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	<b>Pass</b>
<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.	<b>Pass</b>
<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.	<b>Pass</b>
<b>Glycosidase Activity (β-N-Acetylgalactosaminidase)</b> - A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-N-Acetylgalactosaminidase substrate (GalNAcβ1-4Galβ1-4Glc-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.	<b>Pass</b>
<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.	<b>Pass</b>
<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.	<b>Pass</b>
<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.	<b>Pass</b>
<b>Glycosidase Activity (α1-2 Fucosidase)</b> - A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-2Galβ1-4Glc-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.	<b>Pass</b>

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<b>Glycosidase Activity (<math>\alpha</math>1-3 Fucosidase)</b> - A 10 $\mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-3 Galactosidase)</b> - A 10 $\mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-3Gal $\beta$ 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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-3 Mannosidase)</b> - A 10 $\mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Mannosidase substrate (Man $\alpha$ 1-3Man $\beta$ 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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-6 Galactosidase)</b> - A 10 $\mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-6 Mannosidase)</b> - A 10 $\mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Mannosidase substrate (Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>-N-Acetylgalactosaminidase)</b> - A 10 $\mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -N-Acetylgalactosaminidase substrate (GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 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.	<b>Pass</b>
<b>Protease Activity (SDS-PAGE)</b> - A 20 $\mu$ l reaction in 1X Glyco Buffer 2 containing 24 $\mu$ 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.	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> - Remove-iT <sup>®</sup> PNGase F is $\geq$ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>



Authorized by  
Derek Robinson  
26 Feb 2016



Inspected by  
Brad Landgraf  
01 May 2018

