

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Certificate of Analysis

Product Name:	Remove-iT® 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 $\mu$ g of DTT denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 $\mu$ l.
Packaging Lot Number:	10058023
Expiration Date:	11/2020
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® PNGase F Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
P0706LVIAL	Remove-iT® PNGase F	10056702	Pass
B3704SVIAL	10X GlycoBuffer 2	10040324	Pass
B0706SVIAL	10X DTT	10039987	Pass

Assay Name/Specification	Lot # 10058023
Glycosidase Activity ( $\alpha$ 1-3 Mannosidase) A 10 µl 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® PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<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® PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α1-6 Mannosidase) 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® PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass





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<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® PNGase F	Pass
incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	
<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® PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<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® PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<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® PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<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® PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<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® PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Protease Activity (SDS-PAGE)</b> A 20 μl reaction in 1X Glyco Buffer 2 containing 24 μg of a standard mixture of proteins and a minimum of 1,125 units of Remove-iT® 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.	Pass





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Protein Purity Assay (SDS-PAGE) Remove-iT® PNGase F is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
<b>Endoglycosidase F1 Activity</b> A 20 µl reaction in Glyco Buffer 2 containing 20 pmol of flourescently-labeled 2-AA 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.	Pass
<b>Functional Test (Magnetic Beads, Enzyme Removal)</b> Magnetic chitin beads ( 50 $\mu$ I ) were equilibrated and incubated with 1,125 units of Remove-iT® PNGase F in 300 $\mu$ I 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.	Pass
<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.	Pass
<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.	Pass
<b>Glycosidase Activity (<math>\alpha</math>-Glucosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Glucosidase substrate (Glc $\alpha$ 1-6Glc $\alpha$ 1-4Glc-AMC) 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.	Pass
<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(Fucα1-2)Galβ1-4Glc-AMC) 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.	Pass
Glycosidase Activity (α-Neuraminidase)	Pass





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A 10 $\mu$ I reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Neuraminidase substrate (Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) 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.	
<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® PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (α1-3 Fucosidase)</b> A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC) 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.	Pass
Glycosidase Activity (α1-3 Galactosidase) A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-3Galβ1-4GlcNAc-AMC) 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.	Pass

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

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Brad Landgraf Production Scientist 14 Mar 2019

Michae 2.

Michael Tonello Packaging Quality Control Inspector 12 Nov 2019

