

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 #:	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 μ g of DTT denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 μ l.
<i>Lot</i> #:	0031707
Assay Date:	07/2017
Expiration Date:	7/2018
Storage Temp:	$4^{\circ}C$
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 #0031707
Endoglycosidase F1 Activity - 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
Functional Test (Magnetic Beads, Enzyme Removal) - Magnetic chitin beads ($50 \mu l$) 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.	Pass
Glycosidase Activity (Endo F1, F2, H) - 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
Glycosidase Activity (Endo F2, F3) - A 10 μ l 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
Glycosidase Activity (β-Mannosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently- labeled β -Mannosidase substrate (Man β 1-4Man β 1-4Man-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



P0706S/L Lot: 0031707 Page 1 of 3



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Glycosidase Activity (β-Xylosidase) - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently- labeled β -Xylosidase substrate (Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-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
Glycosidase Activity (β1-3 Galactosidase) - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β -Galactosidase substrate (Gal β 1-3GlcNAc β 1-4Gal β 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
Glycosidase Activity (β1-4 Galactosidase) - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β -Galactosidase substrate (Gal β 1-4GlcNAc β 1-3Gal β 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
Glycosidase Activity (β-<i>N</i>-Acetylgalactosaminidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β - <i>N</i> -Acetylgalactosaminidase substrate (GalNAc β 1-4Gal β 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
Glycosidase Activity (β-<i>N</i>-Acetylglucosaminidase) - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β - <i>N</i> -Acetylglucosaminidase substrate (GlcNAc β 1-4GlcNAc β 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
Glycosidase Activity (α-Glucosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently- labeled α -Glucosidase substrate (Glc α 1-6Glc α 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
Glycosidase Activity (α-Neuraminidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently- labeled α -Neuraminidase substrate (Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 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
Glycosidase Activity (α1-2 Fucosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently- labeled α -Fucosidase substrate (Fuc α 1-2Gal β 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



P0706S/L Lot: 0031707 Page 2 of 3



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Assay Name/Specification (minimum release criteria)	Lot #0031707
Glycosidase Activity (α1-3 Fucosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently- labeled α -Fucosidase substrate (Fuc α 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc-AMC) and 450 units of Remove-iT \mathbb{R} 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 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Galactosidase substrate (Gal α 1-3Gal β 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
Glycosidase Activity (α1-3 Mannosidase) - A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Mannosidase substrate (Man α 1-3Man β 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
Glycosidase Activity (α1-6 Galactosidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Galactosidase substrate (Gal α 1-6Gal α 1-6Glc α 1-2Fru-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 (Man α 1-6Man α 1-6(Man α 1-3)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
Glycosidase Activity (α-<i>N</i>-Acetylgalactosaminidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α - <i>N</i> -Acetylgalactosaminidase substrate (GalNAc α 1-3(Fuc α 1-2)Gal β 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
Protease Activity (SDS-PAGE) - 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
Protein Purity Assay (SDS-PAGE) - Remove-iT® PNGase F is \geq 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass

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Authorized by Derek Robinson 26 Feb 2016



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Inspected by Alicia Bielik 23 Jun 2017