

New England Biolabs Product Specification

<i>Product Name:</i>	<i>Remove-iT[®] PNGase F</i>
<i>Catalog #:</i>	<i>P0706S/L</i>
<i>Concentration:</i>	<i>225,000 units/ml</i>
<i>Unit Definition:</i>	<i>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.</i>
<i>Shelf Life:</i>	<i>12 months</i>
<i>Storage Temp:</i>	<i>4°C</i>
<i>Storage Conditions:</i>	<i>50 mM NaCl, 20 mM Tris-HCl, 5 mM EDTA, (pH 7.5 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-P0706S/L v1.0</i>
<i>Effective Date:</i>	<i>26 Feb 2016</i>

Assay Name/Specification (minimum release criteria)

Endoglycosidase F1 Activity - 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[®] PNGase F incubated for 20 hours at 37°C results in no endoglycosidase F1 activity as determined by LC/MS analysis with fluorescent detection.

Functional Test (Magnetic Beads, Enzyme Removal) - 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.

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.

Glycosidase Activity (Endo F2, F3) - 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.

Glycosidase Activity (β-Mannosidase) - 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 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (β-N-Acetylgalactosaminidase) - 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.



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Glycosidase Activity (β -N-Acetylglucosaminidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β -N-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.

Glycosidase Activity (β -Xylosidase) - A 10 μ l 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.

Glycosidase Activity (β 1-3 Galactosidase) - A 10 μ l 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.

Glycosidase Activity (β 1-4 Galactosidase) - A 10 μ l 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.

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.

Glycosidase Activity (α -N-Acetylgalactosaminidase) - A 10 μ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -N-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.

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.

Glycosidase Activity (α 1-2 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.

Glycosidase Activity (α 1-3 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[®] PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

Glycosidase Activity (α 1-3 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.



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Glycosidase Activity (α 1-3 Mannosidase) - A 10 μ l 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.

Glycosidase Activity (α 1-6 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.

Glycosidase Activity (α 1-6 Mannosidase) - A 10 μ l 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.

Protease Activity (SDS-PAGE) - A 20 μ l reaction in 1X Glyco Buffer 2 containing 24 μ g 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.

Protein Purity Assay (SDS-PAGE) - Remove-iT[®] PNGase F is \geq 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.



Date 26 Feb 2016

Derek Robinson
Director of Quality Control

