

New England Biolabs Certificate of Analysis

Product Name: Remove-iT[®] PNGase F
Catalog Number: P0706S
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: 10123406
Expiration Date: 10/2022
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 [®] PNGase F Component List | | | |
|--|---------------------------------|------------|----------------------|
| NEB Part Number | Component Description | Lot Number | Individual QC Result |
| P0706SVIAL | Remove-iT [®] PNGase F | 10123405 | Pass |
| B3704SVIAL | 10X GlycoBuffer 2 | 10118382 | Pass |
| B0706SVIAL | 10X DTT | 10123416 | Pass |

| Assay Name/Specification | Lot # 10123406 |
|---|----------------|
| <p>Glycosidase Activity (α1-6 Galactosidase) 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.</p> | Pass |
| <p>Glycosidase Activity (β-Xylosidase) 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.</p> | Pass |
| <p>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.</p> | Pass |

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|--|----------------|
| <p>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.</p> | Pass |
| <p>Glycosidase Activity (β-N-Acetylglucosaminidase) 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.</p> | Pass |
| <p>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.</p> | Pass |
| <p>Glycosidase Activity (α-Glucosidase) 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[®] PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p> | Pass |
| <p>Glycosidase Activity (α-N-Acetylgalactosaminidase) A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-N-Acetylgalactosaminidase substrate (GalNAcα1-3(Fucaα1-2)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.</p> | Pass |
| <p>Glycosidase Activity (α-Neuraminidase) 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[®] PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p> | Pass |
| <p>Glycosidase Activity (β1-3 Galactosidase) 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.</p> | Pass |

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| <p>Glycosidase Activity (β1-4 Galactosidase) 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.</p> | Pass |
| <p>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.</p> | Pass |
| <p>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.</p> | Pass |
| <p>Protein Purity Assay (SDS-PAGE) Remove-iT[®] PNGase F is \geq 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p> | Pass |
| <p>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.</p> | Pass |
| <p>Glycosidase Activity (α1-2 Fucosidase) A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fuca1-2Galβ1-4Glc-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.</p> | Pass |
| <p>Glycosidase Activity (α1-3 Fucosidase) A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fuca1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-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.</p> | Pass |
| <p>Glycosidase Activity (α1-3 Galactosidase)</p> | Pass |

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| <p>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.</p> | |
| <p>Glycosidase Activity (α1-3 Mannosidase) A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-3Manβ1-4GlcNAc-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.</p> | Pass |
| <p>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.</p> | Pass |
| <p>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.</p> | Pass |

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

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18 Nov 2021



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