

## New England Biolabs Certificate of Analysis

**Product Name:** Remove-iT<sup>®</sup> 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 µg of DTT denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl.  
**Packaging Lot Number:** 10179182  
**Expiration Date:** 01/2024  
**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 <sup>®</sup> PNGase F Component List |                                 |            |                      |
|--|---------------------------------|------------|----------------------|
| NEB Part Number                                | Component Description           | Lot Number | Individual QC Result |
| P0706LVIAL                                     | Remove-iT <sup>®</sup> PNGase F | 10176800   | Pass                 |
| B3704SVIAL                                     | 10X GlycoBuffer 2               | 10118382   | Pass                 |
| B0706SVIAL                                     | 10X DTT                         | 10156824   | Pass                 |

| Assay Name/Specification   | Lot # 10179182 |
|--|----------------|
| <p><b>Endoglycosidase F1 Activity</b><br/>           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.</p>   | Pass           |
| <p><b>Functional Test (Magnetic Beads, Enzyme Removal)</b><br/>           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.</p> | Pass           |
| <p><b>Glycosidase Activity (Endo F1, F2, H)</b><br/>           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.</p>   | Pass           |

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| <p><b>Glycosidase Activity (Endo F2, F3)</b><br/>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>                                 | <b>Pass</b>    |
| <p><b>Glycosidase Activity (α-Glucosidase)</b><br/>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>   | <b>Pass</b>    |
| <p><b>Glycosidase Activity (α-N-Acetylgalactosaminidase)</b><br/>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-N-Acetylgalactosaminidase substrate (GalNAcα1-3(Fuca1-2)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> | <b>Pass</b>    |
| <p><b>Glycosidase Activity (α-Neuraminidase)</b><br/>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>                 | <b>Pass</b>    |
| <p><b>Glycosidase Activity (α1-2 Fucosidase)</b><br/>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>  | <b>Pass</b>    |
| <p><b>Glycosidase Activity (α1-3 Fucosidase)</b><br/>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>                       | <b>Pass</b>    |
| <p><b>Glycosidase Activity (α1-3 Galactosidase)</b><br/>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>                               | <b>Pass</b>    |
| <p><b>Glycosidase Activity (α1-3 Mannosidase)</b></p>  | <b>Pass</b>    |

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|--|----------------|
| <p>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<sup>®</sup> PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>   |                |
| <p><b>Glycosidase Activity (α1-6 Galactosidase)</b><br/>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<sup>®</sup> PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>                    | <b>Pass</b>    |
| <p><b>Glycosidase Activity (α1-6 Mannosidase)</b><br/>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<sup>®</sup> PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>                      | <b>Pass</b>    |
| <p><b>Glycosidase Activity (β-Mannosidase)</b><br/>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.</p>                                  | <b>Pass</b>    |
| <p><b>Glycosidase Activity (β-N-Acetylgalactosaminidase)</b><br/>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.</p>   | <b>Pass</b>    |
| <p><b>Glycosidase Activity (β-N-Acetylglucosaminidase)</b><br/>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.</p> | <b>Pass</b>    |
| <p><b>Glycosidase Activity (β-Xylosidase)</b><br/>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.</p>                             | <b>Pass</b>    |

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

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

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