

## New England Biolabs Certificate of Analysis

**Product Name:** PNGase F  
**Catalog Number:** P0704L  
**Concentration:** 500,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 µg of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl (65 NEB units = 1 IUB milliunit).  
**Packaging Lot Number:** 10094767  
**Expiration Date:** 01/2023  
**Storage Temperature:** -20°C  
**Storage Conditions:** 50 mM NaCl , 20 mM Tris-HCl , 5 mM EDTA , 50 % Glycerol, (pH 7.5 @ 25°C)  
**Specification Version:** PS-P0704S/L v1.0

| PNGase F Component List |                                |            |                      |
|-------------------------|--------------------------------|------------|----------------------|
| NEB Part Number         | Component Description          | Lot Number | Individual QC Result |
| P0704LVIAL              | PNGase F                       | 10094765   | Pass                 |
| B3704SVIAL              | 10X GlycoBuffer 2              | 10091370   | Pass                 |
| B2704SVIAL              | NP-40                          | 10076881   | Pass                 |
| B1704SVIAL              | Glycoprotein Denaturing Buffer | 10090539   | Pass                 |

| Assay Name/Specification   | Lot # 10094767 |
|--|----------------|
| <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 5,000 units of 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><br>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC) and 5,000 units of 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><br>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-4GlcNAcβ1-3Galβ1-4Glc -AMC) and 5,000 units of  | Pass           |

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|--|----------------|
| <p>PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>   |                |
| <p><b>Protease Activity (SDS-PAGE)</b><br/>A 20 µl reaction in 1X Glyco Buffer 2 containing 24 µg of a standard mixture of proteins and a minimum of 10,000 units of 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/>PNGase F is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</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 5,000 units of 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>Endoglycosidase F1 Activity (LC/MS)</b><br/>A 20 µl reaction in Glyco Buffer 2 containing 20 pmoles of 2-AA Man-5 fluorescent standard and 5,000 units of 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>  | <b>Pass</b>    |
| <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 5,000 units of 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 (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 5,000 units of 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 5,000 units of 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>\alpha</math>-N-Acetylgalactosaminidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-N-Acetylgalactosaminidase substrate (GalNAc<math>\alpha</math>1-3(Fuca1-2)Gal<math>\beta</math>1-4Glc-AMC) and 5,000 units of 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>\alpha</math>-Neuraminidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Neuraminidase substrate (Neu5Ac<math>\alpha</math>2-3Gal<math>\beta</math>1-3GlcNAc<math>\beta</math>1-3Gal<math>\beta</math>1-4Glc-AMC) and 5,000 units of 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>\alpha</math>1-2 Fucosidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Fucosidase substrate (Fuca1-2Gal<math>\beta</math>1-4Glc-AMC) and 5,000 units of 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>\alpha</math>1-3 Fucosidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Fucosidase substrate (Fuca1-3Gal<math>\beta</math>1-4GlcNAc<math>\beta</math>1-3Gal<math>\beta</math>1-4Glc-AMC) and 5,000 units of 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>\alpha</math>1-3 Galactosidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Galactosidase substrate (Gal<math>\alpha</math>1-3Gal<math>\beta</math>1-4GlcNAc-AMC) and 5,000 units of 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>\alpha</math>1-3 Mannosidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Mannosidase substrate (Man<math>\alpha</math>1-3Man<math>\beta</math>1-4GlcNAc-AMC) and 5,000 units of 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>\alpha</math>1-6 Galactosidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Galactosidase substrate (Gal<math>\alpha</math>1-6Gal<math>\alpha</math>1-6Glc<math>\alpha</math>1-2Fru-AMC) and 5,000 units of 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>\alpha</math>1-6 Mannosidase)</b></p>   | <b>Pass</b>    |


| Assay Name/Specification   | Lot # 10094767 |
|--|----------------|
| <p>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 5,000 units of 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 (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 5,000 units of 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 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p> | <b>Pass</b>    |

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

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26 Jan 2021



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