

## New England Biolabs Certificate of Analysis

**Product Name:** PNGase F, Recombinant  
**Catalog Number:** P0708S  
**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:** 10137161  
**Expiration Date:** 02/2024  
**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-P0708S/L v1.0

| PNGase F, Recombinant Component List |                                |            |                      |
|--------------------------------------|--------------------------------|------------|----------------------|
| NEB Part Number                      | Component Description          | Lot Number | Individual QC Result |
| P0708SVIAL                           | PNGase F, Recombinant          | 10137160   | Pass                 |
| B3704SVIAL                           | 10X GlycoBuffer 2              | 10118382   | Pass                 |
| B2704SVIAL                           | NP-40                          | 10129282   | Pass                 |
| B1704SVIAL                           | Glycoprotein Denaturing Buffer | 10119058   | Pass                 |

| Assay Name/Specification   | Lot # 10137161 |
|--|----------------|
| <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, Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.       | Pass           |
| <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, Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography. | Pass           |
| <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 5,000 units of PNGase F,  | Pass           |

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|--|----------------|
| <p>Recombinant 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 5,000 units of PNGase F, Recombinant 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 5,000 units of PNGase F, Recombinant 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><br/>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-3Manβ1-4GlcNAc-AMC) and 5,000 units of PNGase F, Recombinant 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 5,000 units of PNGase F, Recombinant 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 5,000 units of PNGase F, Recombinant 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 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, Recombinant 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, Recombinant is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</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 5,000 units of PNGase F, Recombinant 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 5,000 units of PNGase F, Recombinant 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>-Mannosidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-Mannosidase substrate (Man<math>\beta</math>1-4Man<math>\beta</math>1-4Man-AMC) and 5,000 units of PNGase F, Recombinant 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>-Glucosidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Glucosidase substrate (Glc<math>\alpha</math>1-6Glc<math>\alpha</math>1-4Glc-AMC) and 5,000 units of PNGase F, Recombinant 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, Recombinant 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>-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(Fuc<math>\alpha</math>1-2)Gal<math>\beta</math>1-4Glc-AMC) and 5,000 units of PNGase F, Recombinant 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>-N-Acetylglucosaminidase)</b><br/>A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-N-Acetylglucosaminidase substrate (GlcNAc<math>\beta</math>1-4GlcNAc<math>\beta</math>1-4GlcNAc-AMC) and 5,000 units of PNGase F, Recombinant 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>-N-Acetylgalactosaminidase)</b></p>  | <b>Pass</b>    |

| Assay Name/Specification  | Lot # 10137161     |
|---|--------------------|
| <p>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, Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p> <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 5,000 units of PNGase F, Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p> | <p><b>Pass</b></p> |

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

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16 Feb 2022



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