

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Certificate of Analysis

| Product Name:          | PNGase F, Recombinant   |
|------------------------|---|
| Catalog Number:        | P0708L  |
| 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 $\mu$ g of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 $\mu$ l. (65 NEB units = 1 IUB milliunit). |
| Packaging Lot Number:  | 10182244  |
| Expiration Date:       | 03/2025   |
| Storage Temperature:   | -20°C   |
| Storage Conditions:    | 50 mM NaCl , 20 mM Tris-HCl , 5 mM EDTA , 50 % Glycerol, (pH 7.5 @<br>25°C)   |
| Specification Version: | PS-P0708S/L v1.0  |

| PNGase F, Recombinant Component List |                                |            |                      |  |
|--------------------------------------|--------------------------------|------------|----------------------|--|
| NEB Part Number                      | Component Description          | Lot Number | Individual QC Result |  |
| P0708LVIAL                           | PNGase F, Recombinant          | 10180298   | Pass                 |  |
| B3704SVIAL                           | 10X GlycoBuffer 2              | 10148982   | Pass                 |  |
| B2704SVIAL                           | NP-40                          | 10161534   | Pass                 |  |
| B1704SVIAL                           | Glycoprotein Denaturing Buffer | 10153869   | Pass                 |  |

| Assay Name/Specification  | Lot # 10182244 |
|---|----------------|
| <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,<br>F2, H substrate (Dansylated invertase high mannose) and 5,000 units of PNGase F,<br>Recombinant incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography. | Pass           |
| <b>Glycosidase Activity (Endo F2, F3)</b><br>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled Endo F2,<br>F3 substrate (Dansylated fibrinogen biantennary) and 5,000 units of PNGase F,<br>Recombinant incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography.       | Pass           |
| Glycosidase Activity ( $\alpha$ -Glucosidase)<br>A 10 $\mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>$\alpha$ -Glucosidase substrate (Glc $\alpha$ 1-6Glc $\alpha$ 1-4Glc-AMC) and 5,000 units of PNGase F,   | Pass           |





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| Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.  |                |
| <b>Glycosidase Activity (<math>\alpha</math>-N-Acetylgalactosaminidase)</b><br>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>$\alpha$ -N-Acetylgalactosaminidase substrate (GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-AMC) and 5,000<br>units of PNGase F, Recombinant incubated for 20 hours at 37°C results in no<br>detectable activity as determined by thin layer chromatography. | Pass           |
| <b>Glycosidase Activity (α-Neuraminidase)</b><br>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>α-Neuraminidase substrate (Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC) and 5,000<br>units of PNGase F, Recombinant incubated for 20 hours at 37°C results in no<br>detectable activity as determined by thin layer chromatography.   | Pass           |
| <b>Glycosidase Activity (α1-2 Fucosidase)</b><br>A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>α-Fucosidase substrate (Fucα1-2Galβ1-4Glc-AMC) and 5,000 units of PNGase F,<br>Recombinant incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography.  | Pass           |
| <b>Glycosidase Activity (<math>\alpha</math>1-3 Fucosidase)</b><br>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>$\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 5,000 units of<br>PNGase F, Recombinant incubated for 20 hours at 37°C results in no detectable<br>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<br>α-Galactosidase substrate (Galα1-3Galβ1-4GlcNAc-AMC) and 5,000 units of PNGase F,<br>Recombinant incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography.   | Pass           |
| <b>Glycosidase Activity (α1-3 Mannosidase)</b><br>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>α-Mannosidase substrate (Manα1-3Manβ1-4GlcNAc-AMC) and 5,000 units of PNGase F,<br>Recombinant incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography.   | Pass           |
| <b>Glycosidase Activity (α1-6 Galactosidase)</b><br>A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>α-Galactosidase substrate (Galα1-6Galα1-6Glcα1-2Fru-AMC) and 5,000 units of PNGase<br>F, Recombinant incubated for 20 hours at 37°C results in no detectable activity as   | Pass           |





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| determined by thin layer chromatography.   |                |
| <b>Glycosidase Activity (α1-6 Mannosidase)</b><br>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>α-Mannosidase substrate (Manα1-6Manα1-6(Manα1-3)Man-AMC) and 5,000 units of PNGase<br>F, Recombinant incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography.                      | Pass           |
| <b>Glycosidase Activity (β-Mannosidase)</b><br>A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>β-Mannosidase substrate (Manβ1-4Manβ1-4Man-AMC) and 5,000 units of PNGase F,<br>Recombinant incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography.                                  | Pass           |
| <b>Glycosidase Activity (β-N-Acetylgalactosaminidase)</b><br>A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>β-N-Acetylgalactosaminidase substrate (GalNAcβ1-4Galβ1-4Glc-AMC) and 5,000 units of<br>PNGase F, Recombinant incubated for 20 hours at 37°C results in no detectable<br>activity as determined by thin layer chromatography.   | Pass           |
| <b>Glycosidase Activity (β-N-Acetylglucosaminidase)</b><br>A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>β-N-Acetylglucosaminidase substrate (GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC) and 5,000 units<br>of PNGase F, Recombinant incubated for 20 hours at 37°C results in no detectable<br>activity as determined by thin layer chromatography. | Pass           |
| <b>Glycosidase Activity (β-Xylosidase)</b><br>A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>β-Xylosidase substrate (Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC) and 5,000 units of PNGase F,<br>Recombinant incubated for 20 hours at 37°C results in no detectable activity as<br>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<br>β-Galactosidase substrate (Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC) and 5,000 units of<br>PNGase F, Recombinant incubated for 20 hours at 37°C results in no detectable<br>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<br>β-Galactosidase substrate (Galβ1-4GlcNAcβ1-3Galβ1-4Glc -AMC) and 5,000 units of<br>PNGase F, Recombinant incubated for 20 hours at 37°C results in no detectable<br>activity as determined by thin layer chromatography.                | Pass           |





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| <b>Protease Activity (SDS-PAGE)</b><br>A 20 μl reaction in 1X Glyco Buffer 2 containing 24 μg of a standard mixture of<br>proteins and a minimum of 10,000 units of PNGase F, Recombinant incubated for 20<br>hours at 37°C, results in no detectable degradation of the protein mixture as<br>determined by SDS-PAGE with Coomassie Blue detection. | Pass           |
| Protein Purity Assay (SDS-PAGE)<br>PNGase F, Recombinant is ≥ 95% pure as determined by SDS-PAGE analysis using<br>Coomassie Blue detection.   | Pass           |

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

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Maxwell Elkus Production Scientist 21 Feb 2023

The No Michae

Michael Tonello Packaging Quality Control Inspector 28 Feb 2023

