

Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## 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 $\mu$ 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:  | 10231431   |
| Expiration Date:       | 01/2026  |
| 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-P0704S/L v1.0   |

| PNGase F Component List |                                |            |                      |  |
|-------------------------|--------------------------------|------------|----------------------|--|
| NEB Part Number         | Component Description          | Lot Number | Individual QC Result |  |
| P0704LVIAL              | PNGase F                       | 10226043   | Pass                 |  |
| B3704SVIAL              | 10X GlycoBuffer 2              | 10194151   | Pass                 |  |
| B2704SVIAL              | NP-40                          | 10203694   | Pass                 |  |
| B1704SVIAL              | Glycoprotein Denaturing Buffer | 10227027   | Pass                 |  |

| Assay Name/Specification   | Lot # 10231431 |
|--|----------------|
| <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<br>standard and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no<br>endoglycosidase F1 activity as determined by LC/MS analysis with fluorescent<br>detection.                         | 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,<br>F2, H substrate (Dansylated invertase high mannose) and 5,000 units of PNGase F<br>incubated for 20 hours at 37°C results in no detectable activity as determined by<br>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  | Pass           |





Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

| Assay Name/Specification   | Lot # 10231431 |
|--|----------------|
| incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.   |                |
| <b>Glycosidase Activity (<math>\alpha</math>-Glucosidase)</b><br>A 10 µl 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<br>incubated for 20 hours at 37°C results in no detectable activity as determined by<br>thin layer chromatography.   | Pass           |
| <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 incubated for 20 hours at 37°C results in no detectable activity<br>as determined by thin layer chromatography. | Pass           |
| <b>Glycosidase Activity (<math>\alpha</math>-Neuraminidase)</b><br>A 10 $\mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>$\alpha$ -Neuraminidase substrate (Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 5,000<br>units of PNGase F incubated for 20 hours at 37°C results in no detectable activity<br>as determined by thin layer chromatography.     | Pass           |
| <b>Glycosidase Activity (<math>\alpha</math>1-2 Fucosidase)</b><br>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>$\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC) and 5,000 units of PNGase F incubated<br>for 20 hours at 37°C results in no detectable activity as determined by thin layer<br>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 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 Galactosidase)</b><br>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>$\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc-AMC) and 5,000 units of PNGase F<br>incubated for 20 hours at 37°C results in no detectable activity as determined by<br>thin layer chromatography.  | Pass           |
| <b>Glycosidase Activity (<math>\alpha</math>1-3 Mannosidase)</b><br>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>$\alpha$ -Mannosidase substrate (Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC) and 5,000 units of PNGase F<br>incubated for 20 hours at 37°C results in no detectable activity as determined by  | Pass           |





www.neb.com info@neb.com

| Assay Name/Specification  | Lot # 10231431 |
|---|----------------|
| thin layer chromatography.  |                |
| <b>Glycosidase Activity (<math>\alpha</math>1-6 Galactosidase)</b><br>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>$\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-AMC) and 5,000 units of PNGase F<br>incubated for 20 hours at 37°C results in no detectable activity as determined by<br>thin layer chromatography.       | Pass           |
| <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<br>incubated for 20 hours at 37°C results in no detectable activity as determined by<br>thin layer chromatography.   | Pass           |
| <b>Glycosidase Activity (<math>\beta</math>-Mannosidase)</b><br>A 10 $\mu$ I reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>$\beta$ -Mannosidase substrate (Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC) and 5,000 units of PNGase F<br>incubated for 20 hours at 37°C results in no detectable activity as determined by<br>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 incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography.   | Pass           |
| <b>Glycosidase Activity (<math>\beta</math>-N-Acetylglucosaminidase)</b><br>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>$\beta$ -N-Acetylglucosaminidase substrate (GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC) and 5,000 units<br>of PNGase F incubated for 20 hours at 37°C results in no detectable activity as<br>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>incubated for 20 hours at 37°C results in no detectable activity as determined by<br>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 incubated for 20 hours at 37°C results in no detectable activity as   | Pass           |





www.neb.com info@neb.com

| Assay Name/Specification   | Lot # 10231431 |
|--|----------------|
| determined by thin layer chromatography.   |                |
| <b>Glycosidase Activity (<math>\beta</math>1-4 Galactosidase)</b><br>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>$\beta$ -Galactosidase substrate (Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc -AMC) and 5,000 units of<br>PNGase F incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography. | Pass           |
| <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 incubated for 20 hours at 37°C,<br>results in no detectable degradation of the protein mixture as determined by<br>SDS-PAGE with Coomassie Blue detection.  | Pass           |
| Protein Purity Assay (SDS-PAGE)<br>PNGase F is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue<br>detection.  | Pass           |

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Maxwell/Elkus Production Scientist 07 Feb 2024

Hon. M. Michae

Michael Tonello Packaging Quality Control Inspector 14 Feb 2024

