

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:        | 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 $\mu$ 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). |
| Lot Number:            | 10024416  |
| Expiration Date:       | 10/2020   |
| 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 |  |
| P0708SVIAL                           | PNGase F, Recombinant          | 10024413   | Pass                 |  |
| B3704SVIAL                           | 10X GlycoBuffer 2              | 10021262   | Pass                 |  |
| B2704SVIAL                           | NP-40                          | 0161801    | Pass                 |  |
| B1704SVIAL                           | Glycoprotein Denaturing Buffer | 10017111   | Pass                 |  |

| Assay Name/Specification   | Lot # 10024416 |
|--|----------------|
| <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>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 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>Recombinant incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography.     | Pass           |
| Glycosidase Activity (α1-6 Galactosidase)<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   | Pass           |





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| F, Recombinant incubated for 20 hours at 37°C results in no detectable activity as   |                |
| determined by thin layer chromatography.   |                |
| Glycosidase Activity (α1-6 Mannosidase)  | Pass           |
| 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.   |                |
| Glycosidase Activity (β-Mannosidase)   | Pass           |
| 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,<br>Recombinant incubated for 20 hours at 37°C results in no detectable activity as            |                |
| determined by thin layer chromatography.   |                |
| Glycosidase Activity (β-N-Acetylgalactosaminidase)   | Pass           |
| 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.   |                |
| Glycosidase Activity (β-N-Acetylglucosaminidase)   | Pass           |
| 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, Recombinant incubated for 20 hours at 37°C results in no detectable      |                |
| activity as determined by thin layer chromatography.   |                |
| Glycosidase Activity (β-Xylosidase)  | Pass           |
| 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,<br>Recombinant incubated for 20 hours at 37°C results in no detectable activity as      |                |
| determined by thin layer chromatography.   |                |
| Glycosidase Activity (β1-3 Galactosidase)  | Pass           |
| 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, Recombinant incubated for 20 hours at 37 <sup>o</sup> C results in no detectable  |                |
| activity as determined by thin layer chromatography.   |                |
| Glycosidase Activity (β1-4 Galactosidase)  | Pass           |
| 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 PNGase F, Recombinant incubated for 20 hours at 37 <sup>o</sup> C results in no detectable |                |





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| activity as determined by thin layer chromatography.  |                |
| <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           |
| <b>Protein Purity Assay (SDS-PAGE)</b><br>PNGase F, Recombinant is ≥ 95% pure as determined by SDS-PAGE analysis using<br>Coomassie Blue 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>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           |
| <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>Recombinant incubated for 20 hours at 37°C results in no detectable activity as<br>determined by thin layer chromatography. | Pass           |
| <b>Glycosidase Activity (α-N-Acetylgalactosaminidase)</b><br>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled<br>α-N-Acetylgalactosaminidase substrate (GalNAcα1-3(Fucα1-2)Galβ1-4Glc-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           |





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| Glycosidase Activity (α1-2 Fucosidase)   | Pass           |
| A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled                          |                |
| $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-2Gal $\beta$ 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.   |                |
| Glycosidase Activity (α1-3 Fucosidase)   | Pass           |
| A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled                          |                |
| α-Fucosidase substrate (Fucα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-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.   |                |

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

grd

Brad Landgraf Production Scientist 31 Jul 2018

Michae M. 11

Michael Tonello Packaging Quality Control Inspector 06 Nov 2018

