

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 μ g of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 μ l. (65 NEB units = 1 IUB milliunit). |
| Packaging Lot Number: | 10156144 |
| Expiration Date: | 07/2024 |
| 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 | 10156143 | Pass | |
| B3704SVIAL | 10X GlycoBuffer 2 | 10148982 | Pass | |
| B2704SVIAL | NP-40 | 10129282 | Pass | |
| B1704SVIAL | Glycoprotein Denaturing Buffer | 10153869 | Pass | |

| Assay Name/Specification | Lot # 10156144 |
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| Glycosidase Activity (β-Xylosidase) A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β -Xylosidase substrate (Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl β 0 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 |
| Glycosidase Activity (β-N-Acetylgalactosaminidase) 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. | Pass |
| Glycosidase Activity (β-Mannosidase) 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, | Pass |





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| Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography. | |
| Glycosidase Activity (β-N-Acetylglucosaminidase) 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. | Pass |
| Glycosidase Activity (α1-2 Fucosidase) A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-2Galβ1-4Glc-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. | Pass |
| Glycosidase Activity (α1-3 Galactosidase) 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, Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography. | Pass |
| Glycosidase Activity (α1-3 Fucosidase) 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. | Pass |
| Glycosidase Activity (α1-6 Galactosidase) 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. | Pass |
| Glycosidase Activity (α1-3 Mannosidase) 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. | Pass |
| Protease Activity (SDS-PAGE) 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 | Pass |





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| determined by SDS-PAGE with Coomassie Blue detection. | |
| Protein Purity Assay (SDS-PAGE) PNGase F, Recombinant is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | Pass |
| Glycosidase Activity (Endo F2, F3) 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 |
| Glycosidase Activity (Endo F1, F2, H) 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 |
| Glycosidase Activity (α1-6 Mannosidase) 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. | Pass |
| Glycosidase Activity (β1-4 Galactosidase) 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°C results in no detectable activity as determined by thin layer chromatography. | Pass |
| Glycosidase Activity (β1-3 Galactosidase) 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°C results in no detectable activity as determined by thin layer chromatography. | Pass |
| Glycosidase Activity (α-Neuraminidase) A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Neuraminidase substrate (Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-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. | Pass |





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| Glycosidase Activity (α-N-Acetylgalactosaminidase) A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -N-Acetylgalactosaminidase substrate (GalNAc α 1-3(Fuc α 1-2)Gal β 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. | Pass |
| Glycosidase Activity (α-Glucosidase) A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Glucosidase substrate (Glc α 1-6Glc α 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. | Pass |

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

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Alicia Bielik Production Scientist 25 Jul 2022

Josh Hersey

Packaging Quality Control Inspector 25 Jul 2022

