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## New England Biolabs Certificate of Analysis

Product Name: PNGase F (Glycerol-free), Recombinant

Catalog Number: P0709L
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: 10085468
Expiration Date: 10/2022
Storage Temperature: 4°C

Storage Conditions: 50 mM NaCl , 20 mM Tris-HCl , 5 mM EDTA, (pH 7.5 @ 25°C)

Specification Version: PS-P0709S/L v1.0

| PNGase F (Glycerol-free), Recombinant Component List |                                       |            |                      |  |
|--|---------------------------------------|------------|----------------------|--|
| <b>NEB Part Number</b>                               | Component Description                 | Lot Number | Individual QC Result |  |
| P0709LVIAL   | PNGase F (Glycerol-free), Recombinant | 10085469   | Pass                 |  |
| B3704SVIAL   | 10X GlycoBuffer 2                     | 10062315   | Pass                 |  |
| B2704SVIAL   | NP-40                                 | 10076881   | Pass                 |  |
| B1704SVIAL   | Glycoprotein Denaturing Buffer        | 10065640   | Pass                 |  |

| Assay Name/Specification  | Lot # 10085468 |
|---|----------------|
| Glycosidase Activity (α-N-Acetylgalactosaminidase) A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-N-Acetylgalactosaminidase substrate (GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC) and 5000 units of PNGase F (Glycerol-free), 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 5000 units of PNGase F (Glycerol-free), 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 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable   | Pass           |



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| Assay Name/Specification   | Lot # 10085468 |
|--|----------------|
| activity as determined by thin layer chromatography.   | Poor           |
| 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 of PNGase F (Glycerol-free), 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 5000 units of PNGase F (Glycerol-free), 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 5000 units of PNGase F (Glycerol-free), 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 5000 units of PNGase F (Glycerol-free), 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 5000 units of PNGase F (Glycerol-free), 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 5000 units of PNGase F (Glycerol-free), 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 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography. | Pass           |



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| Assay Name/Specification   | Lot # 10085468 |
|--|----------------|
| Assay Name/opecinication   | LOC# 10003400  |
| 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 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography. | Pass           |
| Glycosidase Activity (β-Xylosidase) A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-Xylosidase substrate (Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC) and 5000 units of PNGase F (Glycerol-free), 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 5000 units of PNGase F (Glycerol-free), 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 5000 units of PNGase F (Glycerol-free), 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 (Glycerol-free), 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.                      | Pass           |
| Protein Purity Assay (SDS-PAGE) PNGase F (Glycerol-free), Recombinant is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.   | 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 (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.                  | Pass           |
| Glycosidase Activity (Endo F2, F3)   | Pass           |



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| Assay Name/Specification  | Lot # 10085468 |
|---|----------------|
| A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 5000 units of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.                            |                |
| Glycosidase Activity (α-Glucosidase) A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Glucosidase substrate (Glcα1-6Glcα1-4Glc-AMC) and 5000 units of PNGase F (Glycerol-free), 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** 

23 Oct 2020

Michael Tonello

Packaging Quality Control Inspector

23 Oct 2020



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