## 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 $10 \mu \mathrm{~g}$ of denatured RNase B in 1 hour at $37^{\circ} \mathrm{C}$ in a total reaction volume of $10 \mu \mathrm{l}$ (65 NEB units $=1 \mathrm{IUB}$ milliunit). |
| Packaging Lot Number: | 10085486 |
| Expiration Date: | 10/2022 |
| Storage Temperature: | $-20^{\circ} \mathrm{C}$ |
| Storage Conditions: | $50 \mathrm{mM} \mathrm{NaCl}, 20 \mathrm{mM}$ Tris-HCl , 5 mM EDTA , 50 \% Glycerol, (pH 7.5 @ $25^{\circ} \mathrm{C}$ ) |
| Specification Version: | PS-P0704S/L v1.0 |


| PNGase F Component List | Lot Number | Individual QC Result |  |
| :--- | :--- | :--- | :---: |
| NEB Part Number | Component Description | 10085485 | Pass |
| P0704LVIAL | PNGase F | 10062315 | Pass |
| B3704SVIAL | 10X GlycoBuffer 2 | 10076881 | Pass |
| B2704SVIAL | NP-40 | 10065640 | Pass |
| B1704SVIAL | Glycoprotein Denaturing Buffer |  |  |


| Assay Name/Specification | Lot \# 10085486 |
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| Glycosidase Activity ( $\alpha 1-2$ Fucosidase) <br> A $10 \mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$-Fucosidase substrate (Fuca1-2Gal $\beta 1-4$ GIc-AMC) and 5,000 units of PNGase F incubated for 20 hours at $37^{\circ} \mathrm{C}$ results in no detectable activity as determined by thin layer chromatography. | Pass |
| Glycosidase Activity ( $\alpha$-Neuraminidase) <br> A $10 \mu$ reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$-Neuraminidase substrate (Neu5Aca2-3Gal 1 1-3GIcNAc $\beta 1$-3Galß1-4GIc-AMC) and 5,000 units of PNGase F incubated for 20 hours at $37^{\circ} \mathrm{C}$ results in no detectable activity as determined by thin layer chromatography. | Pass |
| Glycosidase Activity ( $\alpha-\mathrm{N}$-Acetylgalactosaminidase) <br> A $10 \mu$ reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <br> a-N-AcetyIgalactosaminidase substrate (GalNAca1-3(Fuca1-2)Galß1-4GIc-AMC) and 5,000 | Pass |

## Assay Name/Specification as determined by thin layer chromatography. <br> Glycosidase Activity ( $\alpha$-Glucosidase) thin layer chromatography. <br> Glycosidase Activity (Endo F2, F3)

Lot \# 10085486
units of PNGase F incubated for 20 hours at $37^{\circ} \mathrm{C}$ results in no detectable activity

A 10 l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled a-Glucosidase substrate (Glca1-6Glca1-4GIc-AMC) and 5,000 units of PNGase F incubated for 20 hours at $37^{\circ} \mathrm{C}$ results in no detectable activity as determined by

A $10 \mu$ l 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 incubated for 20 hours at $37^{\circ} \mathrm{C}$ results in no detectable activity as determined by thin layer chromatography.

## Glycosidase Activity (Endo F1, F2, H)

A $10 \mu$ l 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 incubated for 20 hours at $37^{\circ} \mathrm{C}$ results in no detectable activity as determined by thin layer chromatography.

## Endoglycosidase F1 Activity (LC/MS)

A $20 \mu \mathrm{l}$ reaction in Glyco Buffer 2 containing 20 pmoles of 2-AA Man-5 fluorescent standard and 5,000 units of PNGase F incubated for 20 hours at $37^{\circ} \mathrm{C}$ results in no endoglycosidase F1 activity as determined by LC/MS analysis with fluorescent detection.

## Glycosidase Activity (a1-6 Galactosidase)

A 10 l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$-Galactosidase substrate (Gala1-6Gala1-6Glca1-2Fru-AMC) and 5,000 units of PNGase F incubated for 20 hours at $37^{\circ} \mathrm{C}$ results in no detectable activity as determined by thin layer chromatography.

## Glycosidase Activity (a1-3 Mannosidase)

A $10 \mu$ reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$-Mannosidase substrate (Mana1-3Manß1-4GlcNAc-AMC) and 5,000 units of PNGase F incubated for 20 hours at $37^{\circ} \mathrm{C}$ results in no detectable activity as determined by thin layer chromatography.

## Glycosidase Activity ( $\alpha 1-3$ Galactosidase)

A $10 \mu \mathrm{l}$ reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$-Galactosidase substrate (Gala1-3Galß1-4GlcNAc-AMC) and 5,000 units of PNGase F incubated for 20 hours at $37^{\circ} \mathrm{C}$ results in no detectable activity as determined by

## Pass

Pass

Pass

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| Assay Name/Specification | Lot \# 10085486 |
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| thin layer chromatography. <br> Glycosidase Activity (a1-3 Fucosidase) <br> A $10 \mu \mathrm{l}$ reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$-Fucosidase substrate (Fuca1-3Gal $\beta 1-4 \mathrm{GlcNAc} \beta 1-3 \mathrm{Gal} 31-4 \mathrm{GIc}-\mathrm{AMC}$ ) and 5,000 units of PNGase F incubated for 20 hours at $37^{\circ} \mathrm{C}$ results in no detectable activity as determined by thin layer chromatography. | Pass |
| Glycosidase Activity (a1-6 Mannosidase) <br> A $10 \mu \mathrm{l}$ reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha-M a n n o s i d a s e ~ s u b s t r a t e ~(M a n \alpha 1-6 M a n \alpha 1-6(M a n \alpha 1-3) M a n-A M C) ~ a n d ~ 5,000 ~ u n i t s ~ o f ~ P N G a s e ~$ F incubated for 20 hours at $37^{\circ} \mathrm{C}$ results in no detectable activity as determined by thin layer chromatography. | Pass |
| Glycosidase Activity ( $\boldsymbol{\beta 1 - 3}$ Galactosidase) <br> A $10 \mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\beta$-Galactosidase substrate (Galß1-3GIcNAc $\beta 1-4$ Gal $\beta 1-4 \mathrm{GIc}-A M C$ ) and 5,000 units of PNGase F incubated for 20 hours at $37^{\circ} \mathrm{C}$ results in no detectable activity as determined by thin layer chromatography. | Pass |
| Glycosidase Activity ( $\beta$-Xylosidase) <br> A $10 \mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\beta$-Xylosidase substrate (Xyl $\beta 1-4$ Xyl $\beta 1-4 X y / \beta 1-4 X y 1-A M C)$ and 5,000 units of PNGase F incubated for 20 hours at $37^{\circ} \mathrm{C}$ results in no detectable activity as determined by thin layer chromatography. | Pass |
| Glycosidase Activity ( $\beta$ - N -AcetyIglucosaminidase) <br> A $10 \mu \mathrm{l}$ reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\beta$-N-Acetylglucosaminidase substrate (GlcNAc $\beta 1-4 \mathrm{GIcNAc} \beta 1-4 \mathrm{GIcNAc}-A M C$ ) and 5,000 units of PNGase F incubated for 20 hours at $37^{\circ} \mathrm{C}$ results in no detectable activity as determined by thin layer chromatography. | Pass |
| Glycosidase Activity ( $\beta$ - N -Acetylgalactosaminidase) <br> A $10 \mu$ reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\beta$-N-Acetylgalactosaminidase substrate (GaINAc $\beta 1-4$ Gal $\beta 1-4 \mathrm{Glc}-\mathrm{AMC}$ ) and 5,000 units of PNGase F incubated for 20 hours at $37^{\circ} \mathrm{C}$ results in no detectable activity as determined by thin layer chromatography. | Pass |
| Glycosidase Activity ( $\beta$-Mannosidase) <br> A $10 \mu$ reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\beta$-Mannosidase substrate (Manß1-4Manß1-4Man-AMC) and 5,000 units of PNGase F incubated for 20 hours at $37^{\circ} \mathrm{C}$ results in no detectable activity as determined by | Pass |


| Assay Name/Specification | Lot \# 10085486 |
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| thin layer chromatography. |  |
| Protein Purity Assay (SDS-PAGE) <br> PNGase F is $\geq 95 \%$ pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | Pass |
| Protease Activity (SDS-PAGE) <br> A $20 \mu$ reaction in 1X Glyco Buffer 2 containing $24 \mu \mathrm{~g}$ of a standard mixture of proteins and a minimum of 10,000 units of PNGase F incubated for 20 hours at $37^{\circ} \mathrm{C}$, results in no detectable degradation of the protein mixture as determined by SDS-PAGE with Coomassie Blue detection. | Pass |
| Glycosidase Activity ( $\beta 1-4$ Galactosidase) <br> A $10 \mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\beta$-Galactosidase substrate (Galß1-4GIcNAc $\beta 1-3 G a / \beta 1-4 G I c-A M C)$ and 5,000 units of PNGase F incubated for 20 hours at $37^{\circ} \mathrm{C}$ 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 28 Nov 2020


