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## New England Biolabs Certificate of Analysis

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

Catalog Number: P0709S
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: 10144177
Expiration Date: 03/2024
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	
P0709SVIAL	PNGase F (Glycerol-free), Recombinant	10144176	Pass	
B3704SVIAL	10X GlycoBuffer 2	10118382	Pass	
B2704SVIAL	NP-40	10129282	Pass	
B1704SVIAL	Glycoprotein Denaturing Buffer	10119058	Pass	

Assay Name/Specification	Lot # 10144177
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) 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.	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 of PNGase F (Glycerol-free), Recombinant incubated for 20 hours at 37°C results in no	Pass



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Assay Name/Specification	Lot # 10144177
detectable activity as determined by thin layer chromatography.	
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 activity as determined by thin layer chromatography.	Pass
Glycosidase Activity ( $\alpha$ -N-Acetylgalactosaminidase) A 10 $\mu$ l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -N-Acetylgalactosaminidase substrate (GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-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-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
<b>Glycosidase Activity (α1-6 Mannosidase)</b> 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 $37^{\circ}$ 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 (α1-6 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 (α1-3 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



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Assay Name/Specification	Lot # 10144177
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
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-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 (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
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 (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



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Assay Name/Specification	Lot # 10144177
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

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

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Alicia Bielik Production Scientist 13 Apr 2022 Josh Hersey Packaging Quality Control Inspector

13 Apr 2022



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