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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 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).

Lot Number: 10043786
Expiration Date: 01/2021
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-P0704S/L v1.0

PNGase F Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
P0704LVIAL	PNGase F	10034948	Pass	
B3704SVIAL	10X GlycoBuffer 2	10040324	Pass	
B2704SVIAL	NP-40	10021820	Pass	
B1704SVIAL	Glycoprotein Denaturing Buffer	10043785	Pass	

Assay Name/Specification	Lot # 10043786
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 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 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	Pass



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incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	
Glycosidase Activity (α 1-6 Mannosidase) A 10 μ I reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Mannosidase substrate (Man α 1-6(Man α 1-3)Man-AMC) and 5,000 units of PNGase F 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 (Fucα1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc-AMC) and 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α 1-2 Fucosidase) A 10 μ I reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Fucosidase substrate (Fuc α 1-2Gal β 1-4Glc-AMC) and 5,000 units of PNGase F 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 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
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 5,000 units of PNGase F 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 5,000 units of PNGase F 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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Assay Name/Specification	Lot # 10043786
incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	
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 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 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Endoglycosidase F1 Activity (LC/MS) A 20 µl 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°C results in no endoglycosidase F1 activity as determined by LC/MS analysis with fluorescent 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 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 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Protein Purity Assay (SDS-PAGE) PNGase F is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	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 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



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Glycosidase Activity (β1-4 Galactosidase) A 10 μ I 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 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 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 5,000 units of PNGase F 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.

Brad Landgraf Production Scientist 09 Nov 2018 Minichiello

Packaging Quality Control Inspector

17 May 2019



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