

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 (Glycerol-free)
Catalog Number:	P0705L
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:	10146861
Expiration Date:	02/2024
Storage Temperature:	4°C
Storage Conditions:	50 mM NaCl , 20 mM Tris-HCl , 5 mM EDTA, (pH 7.5 @ 25°C)
Specification Version:	PS-P0705S/L v1.0

PNGase F (Glycerol-free) Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
P0705LVIAL	PNGase F (Glycerol-free)	10136952	Pass
B3704SVIAL	10X GlycoBuffer 2	10118382	Pass
B2704SVIAL	NP-40	10129282	Pass
B1704SVIAL	Glycoprotein Denaturing Buffer	10119058	Pass

Assay Name/Specification	Lot # 10146861
Glycosidase Activity (α1-3 Mannosidase) A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Mannosidase substrate (Man α 1-3Man β 1-4GlcNAc-AMC) and 5,000 units of PNGase F (Glycerol Free) 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 (Gal α 1-6Gal α 1-6Glc α 1-2Fru-AMC) and 5,000 units of PNGase F (Glycerol Free) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
Glycosidase Activity (α 1-6 Mannosidase) A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Mannosidase substrate (Man α 1-6Man α 1-6(Man α 1-3)Man-AMC) and 5,000 units of PNGase F	Pass





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(Glycerol Free) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	
Glycosidase Activity (α-Glucosidase) A 10 μ I 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 (Glycerol Free) 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 (Glycerol Free) 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 (Glycerol Free) 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 (Glycerol Free) 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 (Glycerol Free) 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 (Glycerol Free) 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 (Glycerol Free) incubated for 20 hours at 37°C results in no detectable activity as	Pass





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determined by thin layer chromatography.	
Endoglycosidase F1 Activity (LC/MS) A 100 µl reaction in Glyco Buffer 2 containing 20 pmoles of 2-AA Man-5 fluorescent standard and 5,000 units of PNGase F (Glycerol-free) incubated for 20 hours at 37°C results in no endoglycosidase F1 activity as determined by LC/MS analysis with fluorescent 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 (Glycerol Free) 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) is \geq 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
Glycosidase Activity (α-Neuraminidase) A 10 μ I reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α -Neuraminidase substrate (Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-AMC) and 5,000 units of PNGase F (Glycerol Free) 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 (Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC) and 5,000 units of PNGase F (Glycerol Free) 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 (Glycerol Free) 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 (Glycerol Free) 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 (β-N-Acetylglucosaminidase) A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β -N-Acetylglucosaminidase substrate (GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC) and 5,000 units of PNGase F (Glycerol Free) 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 (Glycerol-free) 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 (Glycerol Free) 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 18 Apr 2022

Erin Varney

Packaging Quality Control Inspector 18 Apr 2022

