

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
Catalog Number:	P0704S
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 $\mu$ g of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 $\mu$ l (65 NEB units = 1 IUB milliunit).
Packaging Lot Number:	10071840
Expiration Date:	04/2022
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	
P0704SVIAL	PNGase F	10071839	Pass	
B3704SVIAL	10X GlycoBuffer 2	10062315	Pass	
B2704SVIAL	NP-40	10076881	Pass	
B1704SVIAL	Glycoprotein Denaturing Buffer	10065640	Pass	

Assay Name/Specification	Lot # 10071840
<b>Glycosidase Activity (<math>\beta</math>1-4 Galactosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\beta$ -Galactosidase substrate (Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 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
<b>Glycosidase Activity (<math>\beta</math>1-3 Galactosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\beta$ -Galactosidase substrate (Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 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
<b>Glycosidase Activity (β-Xylosidase)</b> 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	Pass





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incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	
<b>Glycosidase Activity (β-N-Acetylglucosaminidase)</b> 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
<b>Glycosidase Activity (β-N-Acetylgalactosaminidase)</b> 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
<b>Glycosidase Activity (<math>\beta</math>-Mannosidase)</b> A 10 $\mu$ I reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\beta$ -Mannosidase substrate (Man $\beta$ 1-4Man $\beta$ 1-4Man-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
<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 5,000 units of PNGase F incubated for 20 hours at 37°C results in no detectable activity as determined by	Pass
thin layer chromatography.	
<b>Glycosidase Activity (<math>\alpha</math>1-6 Galactosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-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
<b>Protein Purity Assay (SDS-PAGE)</b> PNGase F is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
<b>Protease Activity (SDS-PAGE)</b> 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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<b>Glycosidase Activity (Endo F2, F3)</b> 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
<b>Glycosidase Activity (Endo F1, F2, H)</b> 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
<b>Endoglycosidase F1 Activity (LC/MS)</b> 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
<b>Glycosidase Activity (<math>\alpha</math>1-3 Mannosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Mannosidase substrate (Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-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
<b>Glycosidase Activity (α1-3 Galactosidase)</b> 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
<b>Glycosidase Activity (α1-3 Fucosidase)</b> 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 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass
<b>Glycosidase Activity (<math>\alpha</math>1-2 Fucosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-2Gal $\beta$ 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





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Glycosidase Activity (α-Neuraminidase)	Pass
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.	
Glycosidase Activity (α-N-Acetylgalactosaminidase)	Pass
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.	
Glycosidase Activity (α-Glucosidase)	Pass
A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled	
$\alpha$ -Glucosidase substrate (Glc $\alpha$ 1-6Glc $\alpha$ 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.	

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

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Alicia Bielik Production Scientist 30 Jul 2020

Michae

Michael Tonello Packaging Quality Control Inspector 30 Jul 2020

