

## 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 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).  
**Lot Number:** 10013522  
**Expiration Date:** 05/2020  
**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)	10008730	Pass
B3704SVIAL	10X GlycoBuffer 2	0041709	Pass
B2704SVIAL	NP-40	0161801	Pass
B1704SVIAL	Glycoprotein Denaturing Buffer	0161707	Pass

Assay Name/Specification	Lot # 10013522
<b>Protein Purity Assay (SDS-PAGE)</b> PNGase F (Glycerol Free) 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 (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
<b>Glycosidase Activity (α-Neuraminidase)</b> 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 (Glycerol Free) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	Pass

Assay Name/Specification	Lot # 10013522
<p><b>Glycosidase Activity (<math>\alpha</math>-Glucosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Glucosidase substrate (Glc<math>\alpha</math>1-6Glc<math>\alpha</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\alpha</math>-N-Acetylgalactosaminidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-N-Acetylgalactosaminidase substrate (GalNAc<math>\alpha</math>1-3(Fuca1-2)Gal<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\beta</math>1-4 Galactosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-Galactosidase substrate (Gal<math>\beta</math>1-4GlcNAc<math>\beta</math>1-3Gal<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\beta</math>1-3 Galactosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-Galactosidase substrate (Gal<math>\beta</math>1-3GlcNAc<math>\beta</math>1-4Gal<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\alpha</math>1-3 Mannosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Mannosidase substrate (Man<math>\alpha</math>1-3Man<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\alpha</math>1-3 Galactosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Galactosidase substrate (Gal<math>\alpha</math>1-3Gal<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\alpha</math>1-6 Mannosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Mannosidase substrate (Man<math>\alpha</math>1-6Man<math>\alpha</math>1-6(Man<math>\alpha</math>1-3)Man-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.</p>	<b>Pass</b>

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<p><b>Glycosidase Activity (<math>\alpha</math>1-6 Galactosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Galactosidase substrate (Gal<math>\alpha</math>1-6Gal<math>\alpha</math>1-6Glc<math>\alpha</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\beta</math>-N-Acetylgalactosaminidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-N-Acetylgalactosaminidase substrate (GalNAc<math>\beta</math>1-4Gal<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\beta</math>-Mannosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-Mannosidase substrate (Man<math>\beta</math>1-4Man<math>\beta</math>1-4Man-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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\beta</math>-Xylosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-Xylosidase substrate (Xyl<math>\beta</math>1-4Xyl<math>\beta</math>1-4Xyl<math>\beta</math>1-4Xyl-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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\beta</math>-N-Acetylglucosaminidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-N-Acetylglucosaminidase substrate (GlcNAc<math>\beta</math>1-4GlcNAc<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\alpha</math>1-3 Fucosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Fucosidase substrate (Fuca1-3Gal<math>\beta</math>1-4GlcNAc<math>\beta</math>1-3Gal<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (<math>\alpha</math>1-2 Fucosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Fucosidase substrate (Fuca1-2Gal<math>\beta</math>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.</p>	<b>Pass</b>
<p><b>Endoglycosidase F1 Activity (LC/MS)</b></p>	<b>Pass</b>

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<p>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.</p>	
<p><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 (Glycerol Free) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<b>Pass</b>
<p><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 (Glycerol Free) incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<b>Pass</b>

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



Alicia Bielik  
Production Scientist  
23 May 2018



Mary Conlon  
Packaging Quality Control Inspector  
11 Jul 2018