**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 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:** 10189740  
**Expiration Date:** 04/2025  
**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

<table>
<thead>
<tr>
<th>NEB Part Number</th>
<th>Component Description</th>
<th>Lot Number</th>
<th>Individual QC Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0704LVLIAL</td>
<td>PNGase F</td>
<td>10187131</td>
<td>Pass</td>
</tr>
<tr>
<td>B3704SVIAL</td>
<td>10X GlycoBuffer 2</td>
<td>10148982</td>
<td>Pass</td>
</tr>
<tr>
<td>B2704SVIAL</td>
<td>NP-40</td>
<td>10161534</td>
<td>Pass</td>
</tr>
<tr>
<td>B1704SVIAL</td>
<td>Glycoprotein Denaturing Buffer</td>
<td>10181130</td>
<td>Pass</td>
</tr>
</tbody>
</table>

### Assay Name/Specification

<table>
<thead>
<tr>
<th>Assay Name/Specification</th>
<th>Lot # 10189740</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endoglycosidase F1 Activity (LC/MS)</strong></td>
<td>Pass</td>
</tr>
<tr>
<td>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.</td>
<td></td>
</tr>
<tr>
<td><strong>Glycosidase Activity (Endo F1, F2, H)</strong></td>
<td>Pass</td>
</tr>
<tr>
<td>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.</td>
<td></td>
</tr>
<tr>
<td><strong>Glycosidase Activity (Endo F2, F3)</strong></td>
<td>Pass</td>
</tr>
<tr>
<td>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</td>
<td></td>
</tr>
<tr>
<td>Assay Name/Specification</td>
<td>Lot # 10189740</td>
</tr>
<tr>
<td>--------------------------------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Glycosidase Activity (α-Glucosidase)</td>
<td>Pass</td>
</tr>
<tr>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>Glycosidase Activity (α-N-Acetylgalactosaminidase)</td>
<td>Pass</td>
</tr>
<tr>
<td>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-N-Acetylgalactosaminidase substrate (GalNAco1-3(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.</td>
<td></td>
</tr>
<tr>
<td>Glycosidase Activity (α-Neuraminidase)</td>
<td>Pass</td>
</tr>
<tr>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>Glycosidase Activity (α1-2 Fucosidase)</td>
<td>Pass</td>
</tr>
<tr>
<td>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 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</td>
<td></td>
</tr>
<tr>
<td>Glycosidase Activity (α1-3 Fucosidase)</td>
<td>Pass</td>
</tr>
<tr>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>Glycosidase Activity (α1-3 Galactosidase)</td>
<td>Pass</td>
</tr>
<tr>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>Glycosidase Activity (α1-3 Mannosidase)</td>
<td>Pass</td>
</tr>
<tr>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>Assay Name/Specification</td>
<td>Lot # 10189740</td>
</tr>
<tr>
<td>--------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>thin layer chromatography.</td>
<td></td>
</tr>
<tr>
<td><strong>Glycosidase Activity (α1-6 Galactosidase)</strong></td>
<td>Pass</td>
</tr>
<tr>
<td>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 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</td>
<td></td>
</tr>
<tr>
<td><strong>Glycosidase Activity (α1-6 Mannosidase)</strong></td>
<td>Pass</td>
</tr>
<tr>
<td>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 thin layer chromatography.</td>
<td></td>
</tr>
<tr>
<td><strong>Glycosidase Activity (β-Mannosidase)</strong></td>
<td>Pass</td>
</tr>
<tr>
<td>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 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</td>
<td></td>
</tr>
<tr>
<td><strong>Glycosidase Activity (β-N-Acetylgalactosaminidase)</strong></td>
<td>Pass</td>
</tr>
<tr>
<td>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.</td>
<td></td>
</tr>
<tr>
<td><strong>Glycosidase Activity (β-N-Acetylgalactosaminidase)</strong></td>
<td>Pass</td>
</tr>
<tr>
<td>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.</td>
<td></td>
</tr>
<tr>
<td><strong>Glycosidase Activity (β-3 Galactosidase)</strong></td>
<td>Pass</td>
</tr>
<tr>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>Assay Name/Specification</td>
<td>Lot # 10189740</td>
</tr>
<tr>
<td>-----------------------------------------------------------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>determined by thin layer chromatography.</td>
<td></td>
</tr>
<tr>
<td><strong>Glycosidase Activity (β1-4 Galactosidase)</strong></td>
<td>Pass</td>
</tr>
<tr>
<td>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 incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</td>
<td></td>
</tr>
<tr>
<td><strong>Protease Activity (SDS-PAGE)</strong></td>
<td>Pass</td>
</tr>
<tr>
<td>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.</td>
<td></td>
</tr>
<tr>
<td><strong>Protein Purity Assay (SDS-PAGE)</strong></td>
<td>Pass</td>
</tr>
<tr>
<td>PNGase F is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</td>
<td></td>
</tr>
</tbody>
</table>

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit [www.neb.com/trademarks](http://www.neb.com/trademarks) for additional information.

Maxwell Elkus  
Production Scientist  
24 Apr 2023

Michael Tonello  
Packaging Quality Control Inspector  
01 May 2023