

## New England Biolabs Product Specification

<b>Product Name:</b>	<i>PNGase A</i>
<b>Catalog #:</b>	<i>P0707S/L</i>
<b>Concentration:</b>	<i>5,000 units/ml</i>
<b>Unit Definition:</b>	<i>One unit is defined as the amount of enzyme required to remove &gt; 95% of the carbohydrate from 1 µg of denatured recombinant Avidin produced in Maize in 1 hour at 37°C in a total reaction volume of 10 µl.</i>
<b>Shelf Life:</b>	<i>12 months</i>
<b>Storage Temp:</b>	<i>4°C</i>
<b>Storage Conditions:</b>	<i>50 mM NaCl, 20 mM Tris-HCl, 5 mM EDTA, (pH 7.5 @ 25°C)</i>
<b>Specification Version:</b>	<i>PS-P0707S/L v1.0</i>
<b>Effective Date:</b>	<i>27 Sep 2016</i>

### Assay Name/Specification (minimum release criteria)

**Glycosidase Activity (β-Mannosidase)** - A 10 µl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled β-Mannosidase substrate (Manβ1-4Manβ1-4Man-AMC) and 10 units of PNGase A incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity (β-Xylosidase)** - A 10 µl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled β-Xylosidase substrate (Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC) and 10 units of PNGase A incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity (β1-3 Galactosidase)** - A 10 µl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC) and 10 units of PNGase A incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity (β1-4 Galactosidase)** - A 10 µl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC) and 10 units of PNGase A 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 3 containing 1 nM of fluorescently-labeled β-N-Acetylgalactosaminidase substrate (GalNAcβ1-4Galβ1-4Glc-AMC) and 10 units of PNGase A incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity (β-N-Acetylglucosaminidase)** - A 10 µl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled β-N-Acetylglucosaminidase substrate (GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC) and 10 units of PNGase A incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.



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**Glycosidase Activity ( $\alpha$ -Glucosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled  $\alpha$ -Glucosidase substrate (Glc $\alpha$ 1-6Glc $\alpha$ 1-4Glc-AMC) and 10 units of PNGase A incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\alpha$ -Neuraminidase)** - A 10  $\mu$ l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled  $\alpha$ -Neuraminidase substrate (Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 10 units of PNGase A incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\alpha$ 1-2 Fucosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled  $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC) and 10 units of PNGase A incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\alpha$ 1-3 Fucosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled  $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 10 units of PNGase A incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\alpha$ 1-3 Galactosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled  $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc-AMC) and 10 units of PNGase A incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\alpha$ 1-3 Mannosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled  $\alpha$ -Mannosidase substrate (Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC) and 10 units of PNGase A incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\alpha$ 1-6 Galactosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled  $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-AMC) and 10 units of PNGase A incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\alpha$ 1-6 Mannosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled  $\alpha$ -Mannosidase substrate (Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC) and 10 units of PNGase A incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\alpha$ -N-Acetylgalactosaminidase)** - A 10  $\mu$ l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled  $\alpha$ -N-Acetylgalactosaminidase substrate (GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-AMC) and 10 units of PNGase A incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Protease Activity (SDS-PAGE)** - A 20  $\mu$ l reaction in 1X Glyco Buffer 3 containing 24  $\mu$ g of a standard mixture of proteins and a minimum of 25 units of PNGase A 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.



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Assay Name/Specification (minimum release criteria)
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Protein Purity Assay (SDS-PAGE) - PNGase A is $\geq 95\%$ pure as determined by SDS-PAGE analysis using Coomassie Blue detection.
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Date 27 Sep 2016

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Derek Robinson  
Director of Quality Control

