

## New England Biolabs Product Specification

<i>Product Name:</i>	<i><math>\alpha</math>1-6 Mannosidase</i>
<i>Catalog #:</i>	<i>P0727S/L</i>
<i>Concentration:</i>	<i>40,000 units/ml</i>
<i>Unit Definition:</i>	<i>One unit is defined as the amount of enzyme required to cleave &gt; 95% of the terminal <math>\alpha</math>-D-mannose from 1 nmol of Man<math>\alpha</math>1-6Man<math>\alpha</math>1-6Man-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 <math>\mu</math>l.</i>
<i>Shelf Life:</i>	<i>12 months</i>
<i>Storage Temp:</i>	<i>4°C</i>
<i>Storage Conditions:</i>	<i>50 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, (pH 7.5 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-P0727S/L v1.0</i>
<i>Effective Date:</i>	<i>26 Oct 2015</i>

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

**Glycosidase Activity (Endo F1, F2, H)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F1, F2, H substrate (Dansylated invertase high mannose) and 80 units of  $\alpha$ 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity (Endo F2, F3)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 80 units of  $\alpha$ 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity (PNGase F)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled PNGase F substrate (Fluoresceinated fetuin triantennary) and 80 units of  $\alpha$ 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\beta$ -Mannosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled  $\beta$ -Mannosidase substrate (Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC) and 80 units of  $\alpha$ 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\beta$ -N-Acetylgalactosaminidase)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled  $\beta$ -N-Acetylgalactosaminidase substrate (GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC) and 80 units of  $\alpha$ 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\beta$ -N-Acetylglucosaminidase)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled  $\beta$ -N-Acetylglucosaminidase substrate (GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC) and 80 units of  $\alpha$ 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.



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**Glycosidase Activity ( $\beta$ -Xylosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled  $\beta$ -Xylosidase substrate (Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl-AMC) and 80 units of  $\alpha$ 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\beta$ 1-3 Galactosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled  $\beta$ -Galactosidase substrate (Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC) and 80 units of  $\alpha$ 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\beta$ 1-4 Galactosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled  $\beta$ -Galactosidase substrate (Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 80 units of  $\alpha$ 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\alpha$ -Glucosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled  $\alpha$ -Glucosidase substrate (Glc $\alpha$ 1-6Glc $\alpha$ 1-4Glc-AMC) and 80 units of  $\alpha$ 1-6 Mannosidase 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 1 containing 1 nM of fluorescently-labeled  $\alpha$ -N-Acetylgalactosaminidase substrate (GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-AMC) and 80 units of  $\alpha$ 1-6 Mannosidase 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 1 containing 1 nM of fluorescently-labeled  $\alpha$ -Neuraminidase substrate (Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 80 units of  $\alpha$ 1-6 Mannosidase 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 1 containing 1 nM of fluorescently-labeled  $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC) and 80 units of  $\alpha$ 1-6 Mannosidase 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 1 containing 1 nM of fluorescently-labeled  $\alpha$ -Fucosidase substrate (Fuc $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC) and 80 units of  $\alpha$ 1-6 Mannosidase 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 1 containing 1 nM of fluorescently-labeled  $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc-AMC) and 80 units of  $\alpha$ 1-6 Mannosidase 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 1 containing 1 nM of fluorescently-labeled  $\alpha$ -Mannosidase substrate (Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC) and 80 units of  $\alpha$ 1-6 Mannosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.



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

**Glycosidase Activity ( $\alpha$ 1-6 Galactosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled  $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-AMC) and 80 units of  $\alpha$ 1-6 Mannosidase 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 1 containing 24  $\mu$ g of a standard mixture of proteins and a minimum of 400 units of  $\alpha$ 1-6 Mannosidase 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.

**Protein Purity Assay (SDS-PAGE)** -  $\alpha$ 1-6 Mannosidase is  $\geq$  95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.



Date 26 Oct 2015

Derek Robinson  
Director of Quality Control

