

## New England Biolabs Certificate of Analysis

*Product Name:*  $\alpha$ 1-6 Mannosidase  
*Catalog #:* P0727S/L  
*Concentration:* 40,000 units/ml  
*Unit Definition:* One unit is defined as the amount of enzyme required to cleave > 95% of the terminal  $\alpha$ -D-mannose from 1 nmol of Man $\alpha$ 1-6Man $\alpha$ 1-6Man-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10  $\mu$ l.  
*Lot #:* 0091704  
*Assay Date:* 04/2017  
*Expiration Date:* 4/2018  
*Storage Temp:* 4°C  
*Storage Conditions:* 50 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, (pH 7.5 @ 25°C)  
*Specification Version:* PS-P0727S/L v1.0  
*Effective Date:* 19 Feb 2016

Assay Name/Specification (minimum release criteria)	Lot #0091704
<b>Glycosidase Activity (Endo F1, F2, H)</b> - 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.	<b>Pass</b>
<b>Glycosidase Activity (Endo F2, F3)</b> - 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.	<b>Pass</b>
<b>Glycosidase Activity (PNGase F)</b> - 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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\beta</math>-Mannosidase)</b> - 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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\beta</math>-Xylosidase)</b> - 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.	<b>Pass</b>

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Assay Name/Specification (minimum release criteria)	Lot #0091704
<p><b>Glycosidase Activity (<math>\beta</math>1-3 Galactosidase)</b> - A 10 <math>\mu</math>l reaction in Glyco Buffer 1 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 80 units of <math>\alpha</math>1-6 Mannosidase 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 1 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 80 units of <math>\alpha</math>1-6 Mannosidase 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 1 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 80 units of <math>\alpha</math>1-6 Mannosidase 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 1 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 80 units of <math>\alpha</math>1-6 Mannosidase 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>-Glucosidase)</b> - A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Glucosidase substrate (Glc<math>\alpha</math>1-6Glc<math>\alpha</math>1-4Glc-AMC) and 80 units of <math>\alpha</math>1-6 Mannosidase 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>-Neuraminidase)</b> - A 10 <math>\mu</math>l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Neuraminidase substrate (Neu5Ac<math>\alpha</math>2-3Gal<math>\beta</math>1-3GlcNAc<math>\beta</math>1-3Gal<math>\beta</math>1-4Glc-AMC) and 80 units of <math>\alpha</math>1-6 Mannosidase 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 1 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Fucosidase substrate (Fuc<math>\alpha</math>1-2Gal<math>\beta</math>1-4Glc-AMC) and 80 units of <math>\alpha</math>1-6 Mannosidase 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 1 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Fucosidase substrate (Fuc<math>\alpha</math>1-3Gal<math>\beta</math>1-4GlcNAc<math>\beta</math>1-3Gal<math>\beta</math>1-4Glc-AMC) and 80 units of <math>\alpha</math>1-6 Mannosidase 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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Assay Name/Specification (minimum release criteria)	Lot #0091704
<b>Glycosidase Activity (<math>\alpha</math>1-3 Galactosidase)</b> - 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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-3 Mannosidase)</b> - 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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>1-6 Galactosidase)</b> - 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.	<b>Pass</b>
<b>Glycosidase Activity (<math>\alpha</math>-N-Acetylgalactosaminidase)</b> - 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.	<b>Pass</b>
<b>Protease Activity (SDS-PAGE)</b> - 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.	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> - $\alpha$ 1-6 Mannosidase is $\geq$ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>



Authorized by  
Derek Robinson  
19 Feb 2016



Inspected by  
Alicia Bielik  
12 Apr 2017

