

## New England Biolabs Certificate of Analysis

*Product Name:*  $\beta$ -N-Acetylhexosaminidasef  
*Catalog #:* P0721S/L  
*Concentration:* 5,000 units/ml  
*Unit Definition:* One unit is defined as the amount of enzyme required to cleave > 95% of the terminal  $\beta$ -N-acetylgalactosamine from 1 nmol of GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10  $\mu$ l.  
*Lot #:* 0011603  
*Assay Date:* 03/2016  
*Expiration Date:* 3/2018  
*Storage Temp:* -20°C  
*Storage Conditions:* 50 mM NaCl, 20 mM Tris-HCl, 5 mM EDTA, (pH 7.5 @ 25°C)  
*Specification Version:* PS-P0721S/L v1.0  
*Effective Date:* 19 Feb 2016

| Assay Name/Specification (minimum release criteria)   | Lot #0011603 |
|---|--------------|
| <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 50 units of $\beta$ -N-Acetylhexosaminidasef 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 50 units of $\beta$ -N-Acetylhexosaminidasef 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 50 units of $\beta$ -N-Acetylhexosaminidasef 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 50 units of $\beta$ -N-Acetylhexosaminidasef 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 50 units of $\beta$ -N-Acetylhexosaminidasef 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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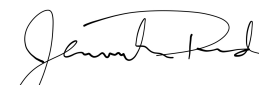
| Assay Name/Specification (minimum release criteria)   | Lot #0011603 |
|---|--------------|
| <b>Glycosidase Activity (<math>\beta</math>1-3 Galactosidase)</b> - A 10 $\mu$ l reaction in Glyco Buffer 1 containing 1 nM of fluorescently-labeled $\beta$ -Galactosidase substrate (Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC) and 50 units of $\beta$ -N-Acetylhexosaminidasef 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>1-4 Galactosidase)</b> - 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 50 units of $\beta$ -N-Acetylhexosaminidasef 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>-Glucosidase)</b> - 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 50 units of $\beta$ -N-Acetylhexosaminidasef 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>-Neuraminidase)</b> - 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 50 units of $\beta$ -N-Acetylhexosaminidasef 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>1-2 Fucosidase)</b> - 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 50 units of $\beta$ -N-Acetylhexosaminidasef 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 Fucosidase)</b> - 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 50 units of $\beta$ -N-Acetylhexosaminidasef 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 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 50 units of $\beta$ -N-Acetylhexosaminidasef 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 50 units of $\beta$ -N-Acetylhexosaminidasef 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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| <p><b>Glycosidase Activity (<math>\alpha</math>1-6 Galactosidase)</b> - A 10 <math>\mu</math>l reaction in Glyco Buffer 1 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 50 units of <math>\beta</math>-N-Acetylhexosaminidasef 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 1 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 50 units of <math>\beta</math>-N-Acetylhexosaminidasef incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>   | <b>Pass</b>  |
| <p><b>Protease Activity (SDS-PAGE)</b> - A 20 <math>\mu</math>l reaction in 1X Glyco Buffer 1 containing 24 <math>\mu</math>g of a standard mixture of proteins and a minimum of 50 units of <math>\beta</math>-N-Acetylhexosaminidasef 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.</p>  | <b>Pass</b>  |
| <p><b>Protein Purity Assay (SDS-PAGE)</b> - <math>\beta</math>-N-Acetylhexosaminidasef is <math>\geq</math> 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>   | <b>Pass</b>  |



Authorized by  
Derek Robinson  
19 Feb 2016



Inspected by  
Jeremiah Read  
16 Mar 2016

