

## New England Biolabs Product Specification

<b>Product Name:</b>	<i>Endo-β-Galactosidase</i>
<b>Catalog #:</b>	P0777S
<b>Concentration:</b>	0.5 mg/ml
<b>Unit Definition:</b>	<i>The enzyme activity is determined by its ability to cleave over 95% of 2 nmol Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC) using less than 10 ng of enzyme within 1 hour at 37°C, in a total reaction volume of 20 μl.</i>
<b>Shelf Life:</b>	24 months
<b>Storage Temp:</b>	-20°C
<b>Storage Conditions:</b>	50 mM Sodium Acetate, 50 mM Sodium Chloride (pH 5.5 @ 25°C)
<b>Specification Version:</b>	PS-P0777S v1.0
<b>Effective Date:</b>	06 Feb 2025

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

**Glycosidase Activity (PNGase F)** - A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled PNGase F substrate (Fluoresceinated fetuin triantennary) and 1 μg of Endo-β-Galactosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity (α-Glucosidase)** - A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled α-Glucosidase substrate (Glcα1-6Glcα1-4Glc-AMC) and 1 μg of Endo-β-Galactosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity (α1-2 Fucosidase)** - A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-2Galβ1-4Glc-AMC) and 1 μg of Endo-β-Galactosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity (α1-3 Galactosidase)** - A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-3Galβ1-4GlcNAc-AMC) and 1 μg of Endo-β-Galactosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity (α1-3 Mannosidase)** - A 10 μl reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-3Manβ1-4GlcNAc-AMC) and 1 μg of Endo-β-Galactosidase 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 3 containing 1 nM of fluorescently-labeled  $\alpha$ -Galactosidase substrate (Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-AMC) and 1  $\mu$ g of Endo- $\beta$ -Galactosidase 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 1  $\mu$ g of Endo- $\beta$ -Galactosidase 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 1  $\mu$ g of Endo- $\beta$ -Galactosidase 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 3 containing 1 nM of fluorescently-labeled  $\beta$ -Mannosidase substrate (Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC) and 1  $\mu$ g of Endo- $\beta$ -Galactosidase incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.

**Glycosidase Activity ( $\beta$ -Xylosidase)** - A 10  $\mu$ l reaction in Glyco Buffer 3 containing 1 nM of fluorescently-labeled  $\beta$ -Xylosidase substrate (Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl-AMC) and 1  $\mu$ g of Endo- $\beta$ -Galactosidase 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 3 containing 1 nM of fluorescently-labeled  $\beta$ -N-Acetylgalactosaminidase substrate (GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC) and 1  $\mu$ g of Endo- $\beta$ -Galactosidase 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 3 containing 1 nM of fluorescently-labeled  $\beta$ -N-Acetylglucosaminidase substrate (GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC) and 1  $\mu$ g of Endo- $\beta$ -Galactosidase 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 2  $\mu$ g of Endo- $\beta$ -Galactosidase 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)** - Endo- $\beta$ -Galactosidase is  $\geq$  95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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Lauren Brown  
Quality Approver

