

## New England Biolabs Certificate of Analysis

**Product Name:** Endo D  
**Catalog Number:** P0742L  
**Concentration:** 50,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 µg of glycosidase-trimmed (trimannosyl core) Fetuin in 1 hour at 37°C in a total reaction volume of 10 µl.  
**Lot Number:** 10053304  
**Expiration Date:** 08/2020  
**Storage Temperature:** 4°C  
**Storage Conditions:** 50 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, (pH 7.5 @ 25°C)  
**Specification Version:** PS-P0742S/L v2.0

Endo D Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
P0742LVIAL	Endo D	10053303	Pass
B3704SVIAL	10X GlycoBuffer 2	10040324	Pass
B0706SVIAL	10X DTT	10039987	Pass

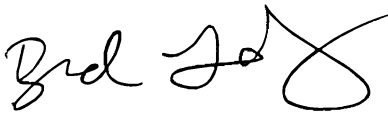
Assay Name/Specification	Lot # 10053304
<p><b>Functional Testing (Magnetic Beads, Enzyme Removal)</b>  Magnetic chitin beads ( 50 µl ) were equilibrated and incubated with 500 units of Endo D in 300 µl of 50 mM ammonium formate, pH 4.4 . The beads were pelleted using a magnetic separation rack. No Endo D was detected in the supernatant as determined by activity assay and mass spectrometry analysis.</p>	Pass
<p><b>Glycosidase Activity (Endo F1, F2, H)</b>  A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled Endo F1, F2, H substrate (Dansylated invertase high mannose) and 500 units of Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (Endo F2, F3)</b>  A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled Endo F2, F3 substrate (Dansylated fibrinogen biantennary) and 500 units of Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass

Assay Name/Specification	Lot # 10053304
<p><b>Glycosidase Activity (<math>\alpha</math>1-3 Mannosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Mannosidase substrate (Man<math>\alpha</math>1-3Man<math>\beta</math>1-4GlcNAc-AMC) and 500 units of Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (<math>\alpha</math>1-6 Galactosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 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 500 units of Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (<math>\alpha</math>1-6 Mannosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 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 500 units of Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (<math>\alpha</math>-N-Acetylgalactosaminidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-N-Acetylgalactosaminidase substrate (GalNAc<math>\alpha</math>1-3(Fuca1-2)Gal<math>\beta</math>1-4Glc-AMC) and 500 units of Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (<math>\beta</math>-Mannosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-Mannosidase substrate (Man<math>\beta</math>1-4Man<math>\beta</math>1-4Man-AMC) and 500 units of Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (<math>\beta</math>-Xylosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\beta</math>-Xylosidase substrate (Xyl<math>\beta</math>1-4Xyl<math>\beta</math>1-4Xyl<math>\beta</math>1-4Xyl-AMC) and 500 units of Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (<math>\alpha</math>-Glucosidase)</b> A 10 <math>\mu</math>l reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled <math>\alpha</math>-Glucosidase substrate (Glc<math>\alpha</math>1-6Glc<math>\alpha</math>1-4Glc-AMC) and 500 units of Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p><b>Glycosidase Activity (<math>\alpha</math>-Neuraminidase)</b></p>	Pass

Assay Name/Specification	Lot # 10053304
<p>A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Neuraminidase substrate (Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC) and 500 units of Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	
<p><b>Glycosidase Activity (α1-2 Fucosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fuca1-2Galβ1-4Glc-AMC) and 500 units of Endo D 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 (α1-3 Fucosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fuca1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC) and 500 units of Endo D 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 (α1-3 Galactosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-3Galβ1-4GlcNAc-AMC) and 500 units of Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> Endo D is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>Glycosidase Activity (β1-3 Galactosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC) and 500 units of Endo D 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 (β1-4 Galactosidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-4GlcNAcβ1-3Galβ1-4Glc -AMC) and 500 units of Endo D 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 (β-N-Acetylgalactosaminidase)</b> A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-N-Acetylgalactosaminidase substrate (GalNAcβ1-4Galβ1-4Glc-AMC) and 500 units of Endo D incubated for 20 hours at 37°C results in no detectable activity as</p>	<b>Pass</b>

Assay Name/Specification	Lot # 10053304
<p>determined by thin layer chromatography.</p> <p><b>Protease Activity (SDS-PAGE)</b> A 20 µl reaction in 1X Glyco Buffer 2 containing 24 µg of a standard mixture of proteins and a minimum of 500 units of Endo D 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>	<p><b>Pass</b></p>

This product has been tested and shown to be in compliance with all specifications.



Brad Landgraf  
Production Scientist  
14 Mar 2019



Michael Tonello  
Packaging Quality Control Inspector  
06 Sep 2019