NEW ENGLAND BioLabs Inc.
be INSPIRED drive DISCOVERY stay GENUINE
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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.*
Packaging Lot Number:	*10084156*
Expiration Date:	*09/2021*
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	10084155	**Pass**
B3704SVIAL	10X GlycoBuffer 2	10062315	**Pass**
B0706SVIAL	10X DTT	10084215	**Pass**

Assay Name/Specification	**Lot # 10084156**
Glycosidase Activity (α1-3 Fucosidase) A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC) and 500 units of Endo D incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**
Glycosidase Activity (α1-3 Galactosidase) 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 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**
Glycosidase Activity (α1-3 Mannosidase) A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-3Manβ1-4GlcNAc-AMC) and 500 units of Endo D incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**

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Assay Name/Specification	Lot # 10084156
Glycosidase Activity (α1-6 Galactosidase) A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Galactosidase substrate (Galα1-6Galα1-6Glcα1-2Fru-AMC) and 500 units of Endo D incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**
Glycosidase Activity (α1-6 Mannosidase) A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Mannosidase substrate (Manα1-6Manα1-6(Manα1-3)Man-AMC) and 500 units of Endo D incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**
Glycosidase Activity (α-N-Acetylgalactosaminidase) A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-N-Acetylgalactosaminidase substrate (GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC) and 500 units of Endo D incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**
Glycosidase Activity (β-Mannosidase) A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-Mannosidase substrate (Manβ1-4Manβ1-4Man-AMC) and 500 units of Endo D incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**
Glycosidase Activity (β-Xylosidase) A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-Xylosidase substrate (Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC) and 500 units of Endo D incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**
Glycosidase Activity (β1-3 Galactosidase) 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 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**
Glycosidase Activity (β1-4 Galactosidase) 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 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**
Glycosidase Activity (β-N-Acetylgalactosaminidase)	**Pass**

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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 37ºC results in no detectable activity as determined by thin layer chromatography.	
Protease Activity (SDS-PAGE) 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.	**Pass**
Protein Purity Assay (SDS-PAGE) Endo D is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**
Functional Testing (Magnetic Beads, Enzyme Removal) 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.	**Pass**
Glycosidase Activity (Endo F1, F2, H) 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 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**
Glycosidase Activity (Endo F2, F3) 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 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**
Glycosidase Activity (α-Glucosidase) A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Glucosidase substrate (Glcα1-6Glcα1-4Glc-AMC) and 500 units of Endo D incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**
Glycosidase Activity (α-Neuraminidase) 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 37ºC results in no detectable activity as	**Pass**

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determined by thin layer chromatography. **Glycosidase Activity (α1-2 Fucosidase)** A 10 µl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled α-Fucosidase substrate (Fucα1-2Galβ1-4Glc-AMC) and 500 units of Endo D incubated for 20 hours at 37ºC results in no detectable activity as determined by thin layer chromatography.	**Pass**

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Alicia Bielik
Production Scientist
11 Nov 2020

Michael Tonello
Packaging Quality Control Inspector
11 Nov 2020