

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: 10143868
Expiration Date: 03/2023
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	10143867	Pass
B3704SVIAL	10X GlycoBuffer 2	10118382	Pass
B0706SVIAL	10X DTT	10084215	Pass

Assay Name/Specification	Lot # 10143868
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
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 37°C 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	Pass

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<p>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>	
<p>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 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p>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 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p>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 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p>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(Fucaα1-2)Galβ1-4Glc-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>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 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p>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 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p>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</p>	Pass

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<p>incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	
<p>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 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p>Glycosidase Activity (α1-3 Fucosidase) 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>	Pass
<p>Glycosidase Activity (α1-2 Fucosidase) 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>	Pass
<p>Glycosidase Activity (β-N-Acetylgalactosaminidase) 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 determined by thin layer chromatography.</p>	Pass
<p>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 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p>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.</p>	Pass
<p>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 37°C results in no detectable activity as determined by thin layer</p>	Pass

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<p>chromatography.</p> <p>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 37°C results in no detectable activity as determined by thin layer chromatography.</p>	<p>Pass</p>

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

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10 Mar 2022



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10 Mar 2022