NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Product Specification

Product Name:	*Endo D*
Catalog #:	*P0742S/L*
Concentration:	*50,000 units/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.*
Shelf Life:	*12 months*
Storage Temp:	*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*
Effective Date:	*05 Jul 2016*

Assay Name/Specification (minimum release criteria)

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.

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.

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 chromatography.

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.

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.

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.

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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 37°C results in no detectable activity as determined by thin layer chromatography.
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.
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.
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.
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 37°C results in no detectable activity as determined by thin layer chromatography.
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 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 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.
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.
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.
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 37°C results in no detectable activity as determined by thin layer chromatography.

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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 37°C 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.
Protein Purity Assay (SDS-PAGE) - Endo D is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

Date 05 Jul 2016

Derek Robinson
Director of Quality Control