NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Certificate of Analysis

Product Name:	*Remove-iT® 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.*
Lot #:	*0011606*
Assay Date:	*06/2016*
Expiration Date:	*6/2017*
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 v1.0*
Effective Date:	*12 Feb 2016*

Assay Name/Specification (minimum release criteria)	**Lot #0011606**
Functional Testing (Magnetic Beads, Enzyme Removal) - Magnetic chitin beads (50 µl) were equilibrated and incubated with 500 units of Remove-iT® Endo D in 300 µl of 50 mM ammonium formate, pH 4.4 . The beads were pelleted using a magnetic separation rack. No Remove-iT® 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 Remove-iT® Endo D incubated for 20 hours at 37°C 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 Remove-iT® Endo D incubated for 20 hours at 37°C 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 Remove-iT® Endo D incubated for 20 hours at 37°C 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 Remove-iT® Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	**Pass**

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Assay Name/Specification (minimum release criteria)	**Lot #0011606**
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 Remove -iT® 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 β-Galactosidase substrate (Galβ1-4GlcNAcβ1-3Galβ1-4Glc -AMC) and 500 units of Remove -iT® Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	**Pass**
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 Remove-iT® Endo D incubated for 20 hours at 37°C 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 Remove-iT® Endo D incubated for 20 hours at 37°C 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 Remove-iT® Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	**Pass**
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 Remove-iT® Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	**Pass**
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 Remove-iT® Endo D incubated for 20 hours at 37°C 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 Remove-iT® Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	**Pass**

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Assay Name/Specification (minimum release criteria)	**Lot #0011606**
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 Remove-iT® Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	**Pass**
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 Remove-iT® Endo D incubated for 20 hours at 37°C 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 Remove -iT® Endo D incubated for 20 hours at 37°C 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 Remove-iT® Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.	**Pass**
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 Remove-iT® 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) - Remove-iT® Endo D is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**

Authorized by
Derek Robinson
12 Feb 2016

Inspected by
Alicia Bielik
30 Jun 2016

nqa ISO 9001 Registered Quality Management
nqa ISO 14001 Registered Environmental Management
nqa ISO 13485 Registered Medical Devices