

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 #: 0011804
Assay Date: 04/2018
Expiration Date: 4/2019
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 #0011804
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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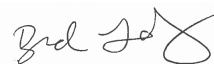
Assay Name/Specification (minimum release criteria)	Lot #0011804
<p>Glycosidase Activity (β1-3 Galactosidase) - A 10 μl reaction in Glyco Buffer 2 containing 1 nM of fluorescently-labeled β-Galactosidase substrate (Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC) and 500 units of Remove-iT[®] Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass
<p>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.</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 Remove-iT[®] 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 Remove-iT[®] 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 Remove-iT[®] 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 (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.</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 (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.</p>	Pass
<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 Remove-iT[®] Endo D incubated for 20 hours at 37°C results in no detectable activity as determined by thin layer chromatography.</p>	Pass

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Assay Name/Specification (minimum release criteria)	Lot #0011804
<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 Remove-iT[®] 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 Remove-iT[®] 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 Remove-iT[®] 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(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.</p>	Pass
<p>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.</p>	Pass
<p>Protein Purity Assay (SDS-PAGE) - Remove-iT[®] Endo D is \geq 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass



Authorized by
Derek Robinson
12 Feb 2016



Inspected by
Brad Landgraf
17 Apr 2018

