

Remove-iT[®] Endo D



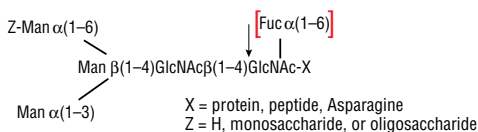
P0742S



1,500 units **50,000 U/ml** **Lot: 0011406**
Store at 4°C **Exp: 6/15**

Description: Remove-iT Endo D, also known as Endoglycosidase D, is a recombinant glycosidase, which cleaves within the chitobiose core of paucimannose N-linked glycans, with or without extensions in the antennae. Remove-iT Endo D is tagged with a chitin binding domain (CBD) for easy removal from a reaction, and is supplied glycerol-free for optimal performance in HPLC- and MS- intensive methods.

Specificity:



Detailed Specificity:

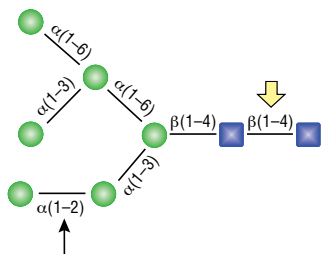


Figure 1: Detailed specificity of Remove-iT Endo D. The reaction contained 0.08 mU of α -1-2 Mannosidase (Prozyme #GKX-5009), 50 units of Remove-iT Endo D and 2 μ l 0.5 M NaOAc pH 5.0 buffer in a total reaction volume of 10 μ l. Reactions were incubated at 37°C for 2 hours. Following removal of the bottom branch, Remove-iT Endo D is active on a complex upper arm (1).

Source: A truncated Endo D gene cloned from *Streptococcus pneumoniae* and expressed in *E. coli* as a fusion to chitin binding domain (2)

Supplied in: 20 mM Tris-HCl (pH 7.5 @ 25°C), 50 mM NaCl, and 1 mM Na₂EDTA

Reagents Supplied with Enzyme:

10X G7 Reaction Buffer
(0.5 M Sodium Phosphate, pH 7.5 @ 25°C)
10X DTT (0.4 M DTT)

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 μ g of glycosidase-trimmed (trimannosyl core) Fetuin in 1 hour at 37°C in a total reaction volume of 10 μ l.

Unit Definition Assay: 10 μ g of glycosidase-trimmed (trimannosyl core) Fetuin are denatured with 1X DTT at 95°C for 3 minutes. After the addition of 1X G7 Reaction Buffer, two-fold dilutions of Remove-iT Endo D are added and the reaction mix is incubated for 1 hour at 37°C. Separation of reaction products is visualized by SDS-PAGE.

Molecular Weight: 140,000 Daltons

Quality Assurance: No contaminating exoglycosidase or endoglycosidase activity could be detected. No contaminating proteolytic activity could be detected.

Quality Controls

Glycosidase Assays: 500 units of Remove-iT Endo D were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides in a 10 μ l reaction for 20 hours at 37°C. The reaction products were analyzed by TLC for digestion of substrate.

No other glycosidase activities were detected (ND) with the following substrates:

β -N-Acetylgalactosaminidase:
GalNAc β 1-4Gal β 1-4Glc-AMC ND

α -N-Acetylgalactosaminidase:
GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc-AMC ND

α -Fucosidase:
Fuc α 1-2Gal β 1-4Glc-AMC ND
Gal β 1-4 (Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND

β -Galactosidase:
Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC ND
Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc-AMC ND

α -Galactosidase:
Gal α 1-3Gal β 1-4Gal-AMC ND
Gal α 1-6Gal α 1-6Glc α 1-2Fru-AMC ND

α -Neuraminidase:
Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-AMC ND

α -Mannosidase:
Man α 1-3Man β 1-4GlcNAc-AMC ND
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

β -Glucosidase:
Glc β 1-4Glc β 1-4Glc-AMC ND

α -Glucosidase:
Glc α 1-6Glc α 1-4Glc-AMC ND

β -Xylosidase:
Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-AMC ND

β -Mannosidase:
Man β 1-4Man β 1-4Man-AMC ND

Endo F₁, F₂, H:
Dansylated invertase high mannose ND

Endo F₂, F₃:
Dansylated fibrinogen biantennary ND

Protease Assay: After incubation of 500 units of Remove-iT Endo D with 0.2 nmol of a standard mixture of proteins in a 20 μ l reaction for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

Magnetic Chitin Beads Assay: 500 units of Remove-iT Endo D were bound to a 50 μ l slurry of magnetic chitin beads and washed with 300 μ l of 50 mM ammonium formate, pH 4.4. No Remove-iT Endo D was detected in the flow-through, as determined by activity assay and mass spectrometry analysis.

Heat Inactivation: 65°C for 10 minutes

Reaction Conditions: Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate. Typical reaction conditions are as follows:

- Combine 10–20 μ g of glycoprotein, 1 μ l of 10X DTT and H₂O (if necessary) to make a 10 μ l total reaction volume.
- Denature the glycoprotein by heating the reaction at 55°C for 10 minutes.
- Make a total reaction volume of 20 μ l by adding 2 μ l 10X G7 Reaction Buffer, H₂O and 1–5 μ l Remove-iT Endo D.
- Incubate the reaction at 37°C for 1 hour.

Remove-iT Endo D Removal Magnetic Chitin Bead Protocol:

Materials:

Remove-iT Endo D (NEB #P0742)
Chitin Magnetic Beads (NEB #E8036)
Magnetic Separation Rack (NEB #S1506 or NEB #S1509)

- Pipette 50 μ l Chitin Magnetic Beads into an eppendorf tube and place the eppendorf in a magnetic separation rack. Let the magnet attract the chitin beads, then pipette off the liquid supernatant and discard the supernatant.
- With the eppendorf on the magnetic separation rack, wash the magnetic chitin beads 2 x 500 μ l with 50 mM NH₄ Formate pH 4.4 (or buffer of choice). Pipette off the supernatant and discard.
- Add the deglycosylated glycoprotein sample into the eppendorf with magnetic chitin beads.
- Rock the deglycosylated glycoprotein sample with the magnetic chitin beads for 10 minutes at 4°C.
- Place the eppendorf back on the magnetic separation rack, and allow the magnet to attract the chitin beads. Pipette off the supernatant and keep.
- Wash the magnetic chitin beads 3 x 100 μ l with 50 mM NH₄ Formate, pH 4.4 (or buffer of choice). Pipette off the supernatant from each wash and keep.
- Combine all supernatants from steps 5 & 6, as these contain the deglycosylated glycoprotein.
- Analyze sample by method of choice

Notes on Use:

- To deglycosylate a native glycoprotein, longer incubation time, as well as more enzyme, may be required.
- Remove-iT Endo D is not recommended for use with Glycoprotein Denaturing Buffer containing both SDS and DTT, as Remove-iT Endo D is inhibited by SDS and, unlike other endoglycosidases, NP-40 does not counteract the SDS inhibition.
- Removal of Remove-iT Endo D from the deglycosylation reaction can be scaled up linearly with larger amounts of chitin magnetic beads.
- Chitin Magnetic Beads Binding Capacity is 0.4 μ g/ μ l of CBD-tagged protein.

(see other side)

References:

1. McLeod, E., Shi, S. and Magnelli, P., New England Biolabs, Inc., unpublished results.
2. McLeod, E., New England Biolabs, Inc., unpublished results.

Companion Products:

Chitin Magnetic Beads

#E8036S 5 ml

#E8036L 25 ml

6-Tube Magnetic Separation Rack

#S1506S 6 tubes

12-Tube Magnetic Separation Rack

#S1509S 12 tubes



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