Remove-iT™ Endo D

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P0742S 001130314031

P0742S

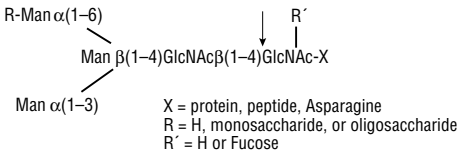
RR 37° 65°

1,500 units **50,000 U/ml** **Lot: 0011303**

Store at 4°C **Exp: 3/14**

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:

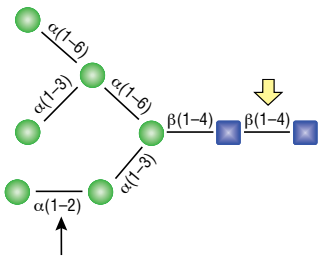


Figure 1: Detailed specificity of Remove-iT Endo D. *The reaction contained 0.08 mU of α1-2 Mannosidase (Prozyme #GKX-5009), 50 units of Remove-iT Endo D and 2 µl 0.5 M NaOAc pH 5.0 buffer in a total reaction volume of 10 µl. Reactions were incubated at 37°C for 2 hours. Following removal of the bottom 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 Na_2EDTA

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 µg of glycosidase-trimmed (trimannosyl core) Fetuin in 1 hour at 37°C in a total reaction volume of 10 µl.

Unit Definition Assay: 10 µg 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 µl 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:
GalNAcβ1-4Galβ1-4Glc-AMC ND

α-*N*-Acetylgalactosaminidase:
GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC ND

α-Fucosidase:
Fucα1-2Galβ1-4Glc-AMC ND
Galβ1-4 (Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

β-Galactosidase:
Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND
Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC ND

α-Galactosidase:
Galα1-3Galβ1-4Gal-AMC ND
Galα1-6Galα1-6Glcα1-2Fru-AMC ND

α-Neuraminidase:
Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC ND

α-Mannosidase:
Manα1-3Manβ1-4GlcNAc-AMC ND
Manα1-6Manα1-6(Manα1-3)Man-AMC ND

β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-AMC ND

α-Glucosidase:
Glcα1-6Glcα1-4Glc-AMC ND

β-Xylosidase:
Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC ND

β-Mannosidase:
Manβ1-4Manβ1-4Man-AMC ND

Endo F_1, F_2, H:
Dansylated invertase high mannose ND

Endo F_2, F_3:
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 µl 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 µl slurry of magnetic chitin beads and washed with 300 µl 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:

1. Combine 10–20 µg of glycoprotein, 1 µl of 10X DTT and H_20 (if necessary) to make a 10 µl total reaction volume.
2. Denature the glycoprotein by heating the reaction at 95°C for 3 minutes.
3. Make a total reaction volume of 20 µl by adding 2 µl 10X G7 Reaction Buffer, H_20 and 1–5 µl Remove-iT Endo D.
4. 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)

1. Pipette 50 µl 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.
2. With the eppendorf on the magnetic separation rack, wash the magnetic chitin beads 2 x 500 µl with 50 mM NH_4 Formate pH 4.4 (or buffer of choice). Pipette of the supernatant and discard.
3. Add the deglycosylated glycoprotein sample into the eppendorf with magnetic chitin beads.
4. Rock the deglycosylated glycoprotein sample with the magnetic chitin beads for 10 minutes at 4°C.
5. Place the eppendorf back on the magnetic separation rack, and allow the magnet to attract the chitin beads. Pipette off the supernatant and keep.
6. Wash the magnetic chitin beads 3 x 100 µl with 50 mM NH_4 Formate, pH 4.4 (or buffer of choice). Pipette off the supernatant from each wash and keep.
7. Combine all supernatants from steps 5 & 6, as these contain the deglycosylated glycoprotein.
8. 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 µg/µl of CBD-tagged protein.

(see other side)

CERTIFICATE OF ANALYSIS

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