

# Remove-iT<sup>®</sup> Endo D



## P0742S

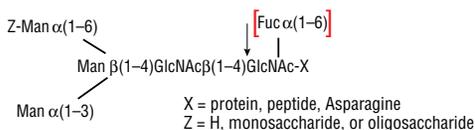


**1,500 units**    **50,000 U/ml**    **Lot: 0011310**

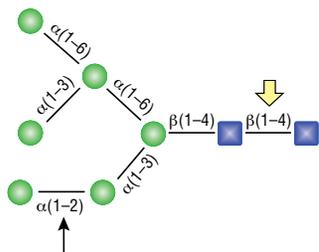
**Store at 4°C**    **Exp: 10/14**

**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  $\alpha$ -1-2 Mannosidase (Prozyme #GKX-5009), 50 units of Remove-iT Endo D and 2  $\mu$ l 0.5 M NaOAc pH 5.0 buffer in a total reaction volume of 10  $\mu$ 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 Na<sub>2</sub>EDTA

### 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  $\mu$ g of glycosidase-trimmed (trimannosyl core) Fetuin in 1 hour at 37°C in a total reaction volume of 10  $\mu$ l.

**Unit Definition Assay:** 10  $\mu$ 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  $\mu$ 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:

**$\beta$ -N-Acetylgalactosaminidase:** GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC ND

**$\square$ -N-Acetylgalactosaminidase:** GalNAc $\square$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Fucosidase:** Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC ND  
Gal $\beta$ 1-4 (Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC ND

**$\beta$ -Galactosidase:** Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC ND  
Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\square$ 1-4Glc-AMC ND

**$\square$ -Galactosidase:** Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal-AMC ND  
Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-AMC ND

**$\alpha$ -Neuraminidase:** Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Mannosidase:** Man $\alpha$ 1-3Man $\square$ 1-4GlcNAc-AMC ND  
Man $\alpha$ 1-6Man $\square$ 1-6(Man $\square$ 1-3)Man-AMC ND

**$\beta$ -Glucosidase:** Glc $\beta$ 1-4Glc $\beta$ 1-4Glc-AMC ND

**$\square$ -Glucosidase:** Glc $\square$ 1-6Glc $\square$ 1-4Glc-AMC ND

**$\beta$ -Xylosidase:** Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl-AMC ND

**$\beta$ -Mannosidase:** Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC ND

**Endo F<sub>1</sub>, F<sub>2</sub>, H:** Dansylated invertase high mannose ND

**Endo F<sub>2</sub>, F<sub>3</sub>:** 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  $\mu$ 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  $\mu$ l slurry of magnetic chitin beads and washed with 300  $\mu$ 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  $\mu$ g of glycoprotein, 1  $\mu$ l of 10X DTT and H<sub>2</sub>O (if necessary) to make a 10  $\mu$ l total reaction volume.
- Denature the glycoprotein by heating the reaction at 55°C for 10 minutes.
- Make a total reaction volume of 20  $\mu$ l by adding 2  $\mu$ l 10X G7 Reaction Buffer, H<sub>2</sub>O and 1–5  $\mu$ 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  $\mu$ 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  $\mu$ l with 50 mM NH<sub>4</sub> 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  $\mu$ l with 50 mM NH<sub>4</sub> 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  $\mu$ g/ $\mu$ 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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