

Remove-iT™ Endo S



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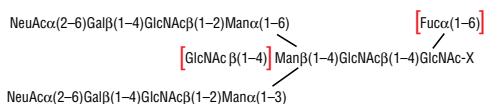


6,000 units 200,000 U/ml Lot: 0021301

Store at 4°C Exp: 1/14

Description: Remove-iT Endo S is an endoglycosidase with a uniquely high specificity for removing *N*-linked glycans from the chitobiose core of the heavy chain of native IgG (1). Remove-iT Endo S 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: X = protein, peptide, Asparagine, or free glycan, as Remove-iT Endo S does not have a strict peptide requirement for activity. Remove-iT Endo S is active on a substrate with or without core $\alpha(1-6)$ fucosylation as well as a with or without a bisecting *N*-acetylglucosamine. Triantennary and tetrantennary sialylated or asialo glycans are not a substrate for Remove-iT Endo S (2).

Source: Remove-iT Endo S is cloned from *Streptococcus pyogenes* (1) and overexpressed as a fusion to the chitin binding domain in *E. coli* (3).

Supplied in: 20 mM Tris-HCl (pH 7.5 @ 25°C), 50 mM NaCl, and 5 mM Na₂EDTA

Reagents Supplied with Enzyme:

10X G6 Reaction Buffer
(0.5 M Sodium Acetate, pH 5.5 @ 25°C
50 mM CaCl₂)

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 5 μ g of native mouse monoclonal IgG in 1 hour at 37°C in a total reaction volume of 10 μ l

Unit Definition Assay: 5 μ g of IgG in 1X G6 Reaction Buffer are incubated with two-fold dilutions of Remove-iT Endo S for 1 hour at 37°C. Separation of reaction products is visualized by SDS-PAGE.

Molecular Weight: 136,000 Daltons

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

Quality Controls

Glycosidase Assays: 2,000 units of Remove-iT Endo S 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

Protease Assay: After incubation of 1,400 units of Remove-iT Endo S 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: 2,000 units of Remove-iT Endo S 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 S was detected in the flow through as determined by activity assay and mass spectrometry analysis.

Heat Inactivation: 55°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 100 μ g of native IgG, 1 μ l of 10X G6 Reaction Buffer and H₂O (if necessary) to make a 10 μ l total reaction volume.
- Add 1 μ l Remove-iT Endo S.
- Incubate reaction at 37°C for 1 hour.

Remove-iT Endo S Removal Magnetic Chitin Bead Protocol:

Materials:

Remove-iT Endo S (NEB #P0741)
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.
- 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 of 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 of the supernatant from each wash and keep.
- Combine all supernatants from steps 5 & 6, as these are the deglycosylated glycoprotein.
- Analyze sample by method of choice

Notes on Use:

- Recommended storage temperature is 4°C, avoid repeat freeze-thaw cycles.
- Removal of Remove-iT Endo S from the deglycosylation reaction can be scaled up linearly with larger volumes of chitin magnetic beads.
- Chitin Magnetic Beads Binding Capacity is 0.4 μ g/ μ l of CBD-tagged protein.

References:

- Collin, M. and Olsén, A. *The EMBO Journal* (2001) 20, 3046–3055.
- Bielik, A., McLeod, E. and Magnelli, P., New England Biolabs, Inc., unpublished results.
- McLeod, E., New England Biolabs, Inc., unpublished results.

Companion Products:

Chitin Magnetic Beads
#E8036S 20 ml
#E8036L 100 ml

6-Tube Magnetic Separation Rack
#S1506S 6 tubes

12-Tube Magnetic Separation Rack
#S1509S 12 tubes

Endoglycosidase Reaction Buffer Pack
#B0701S 4 x 1 ml



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