

# Remove-iT<sup>®</sup> Endo S



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P0741S 002140815081

## P0741S

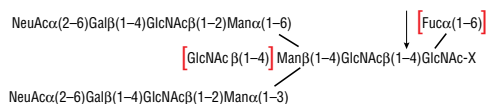


6,000 units    200,000 U/ml    Lot: 0021408

Store at 4°C    Exp: 8/15

**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 tetraantennary 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 Na<sub>2</sub>EDTA

### Reagents Supplied with Enzyme:

10X G6 Reaction Buffer  
(0.5 M Sodium Acetate, pH 5.5 @ 25°C  
50 mM CaCl<sub>2</sub>)

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

**Unit Definition Assay:** 5  $\mu$ 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  $\mu$ 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

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

**$\alpha$ -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 $\beta$ 1-4GlcNAc-AMC    ND  
Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC    ND

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

**$\alpha$ -Glucosidase:**  
Glc $\alpha$ 1-6Glc $\alpha$ 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

**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  $\mu$ 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:** 2,000 units of Remove-iT Endo S 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 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  $\mu$ g of native IgG, 1  $\mu$ l of 10X G6 Reaction Buffer and H<sub>2</sub>O (if necessary) to make a 10  $\mu$ l total reaction volume.
- Add 1  $\mu$ 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  $\mu$ 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.
- 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 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  $\mu$ l with 50 mM NH<sub>4</sub> 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  $\mu$ g/ $\mu$ 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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