Remove-iT® Endo S

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P0741S 002150116011

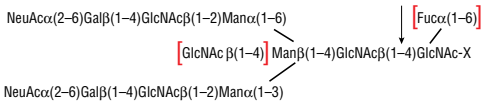
P0741S

6,000 units **200,000 U/ml** **Lot: 0021501**

Store at 4°C **Exp: 1/16**

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 α(1-6)fucosylation as well as a with or without a bisecting *N*-acetylglucosamine. Triantennary and tetrantennary sialyated 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_2EDTA

Reagents Supplied with Enzyme:
10X GlycoBuffer 1:
[0.5 M Sodium Phosphate (pH 7.5 @ 25°C)]

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

New Reaction Buffer

Unit Definition Assay: 5 µg of IgG in 1X GlycoBuffer 2 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 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

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

1. Combine 100 µg of native IgG, 1 µl of 10X GlycoBuffer 2 and H_2O (if necessary) to make a 10 µl total reaction volume.
2. Add 1 µl Remove-iT Endo S.
3. 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)

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.
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 of the supernatant from each wash and keep.
7. Combine all supernatants from steps 5 & 6, as these are the deglycosylated glycoprotein.
8. 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 µg/µl of CBD-tagged protein.

References:

1. Collin, M. and Olsén, A. *The EMBO Journal* (2001) 20, 3046–3055.
2. Bielik, A., McLeod, E. and Magnelli, P., New England Biolabs,Inc., unpublished results.
3. 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

ISO 9001 Registered Quality Management
ISO 14001 Registered Environmental Management
ISO 13485 Registered Medical Devices

CERTIFICATE OF ANALYSIS