Unit Definition Assay: 5 μg 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 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:**

**α–N-Acetylgalactosaminidase:**
GalNAccα[1–3](Fucα[1–2])Galβ[1–4]Glc-AMC ND

**α-Fucosidase:**

**β-Galactosidase:**

**α-Galactosidase:**

**α–Neuraminidase:**

**α–Mannosidase:**

**β-Glucosidase:**

**α-Glucosidase:**

**β-Xylosidase:**

**β-Mannosidase:**

Endo Fα, Fβ, Hα:
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 G6 Reaction Buffer and H2O (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 #S1509S)
- Magnetic Chitin Beads (NEB #E8036L or #E8036)

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 NH4 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 NH4, 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:

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

NEW ENGLAND BIOLABS® and REMOVE-IT® are registered trademarks of New England Biolabs, Inc.