Remove-iT™ PNGase F

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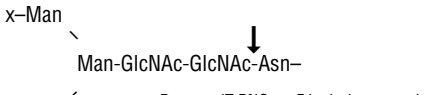
P0706S

37° Yes

6,750 units **225,000 U/ml** **Lot: 0031310**
Store at 4°C **Exp: 10/14**

Description: Remove-iT PNGase F is an amidase that cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides from *N*-linked glycoproteins (1). Remove-iT PNGase F 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:



Remove-iT PNGase F hydrolyzes nearly all types of *N*-glycan chains from glycopeptides/proteins. [x = H or sugar(s)]

Source: Remove-iT PNGase F is purified from *Flavobacterium meningosepticum* (2).

Supplied in: 20 mM Tris-HCl (pH 7.5 @ 25°C), 50 mM NaCl and 5 mM Na_2EDTA

Reagents Supplied with Enzyme:
10X DTT
(0.4 M DTT)

10X G7 Reaction Buffer
(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 DTT denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl

Unit Definition Assay: 5 µg of RNase B are denatured with 1X DTT at 55°C for 10 minutes. After the addition of 1X G7 Reaction Buffer, two-fold dilutions of Remove-iT PNGase F 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: 41,000 Daltons

Quality Assurance: No contaminating exoglycosidase or Endoglycosidase F1, F2 or F3 activity could be detected. No contaminating proteolytic activity could be detected.

Quality Controls

Glycosidase Assays: 5,000 units of Remove-iT PNGase F 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

Endo F_2, F_3:
Dansylated fibrinogen biantennary. ND

Protease Assay: After incubation of 5,000 units of Remove-iT PNGase F 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.

Endoglycosidase F1 Assay: After incubation of 5,000 units of Remove-iT PNGase F with 20 pmol of 2-AA Man-5 fluorescent standard, for 20 hours at 37°C, no endoglycosidase F1 activity could be detected by LC/MS analysis with fluorescence detection.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

Magnetic Chitin Beads Assay: 5,000 units of Remove-iT PNGase F 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 PNGase F was detected in the flow through as determined by activity assay and mass spectrometry analysis.

Heat Inactivation: 75°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 10–20 µg of glycoprotein, 1 µl of 10X DTT and H_20 (if necessary, to make a 10 µl total reaction volume).
2. Denature glycoprotein by heating reaction at 55°C for 10 minutes.
3. Make a total reaction volume of 20 µl by adding 2 µl 10X G7 Reaction Buffer, H_20 and 1–5 µl Remove-iT PNGase F.
4. Incubate reaction at 37°C for 1 hour.

Remove-iT PNGase F Magnetic Chitin Bead Protocol:
Materials.

Remove-iT PNGase F (NEB #P0706)

Chitin Magnetic Beads (NEB #E8036)

Magnetic Separation Rack (NEB #S1506, 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:

- To deglycosylate a native glycoprotein, longer incubation time, as well as more enzyme, may be required.
- Using typical RNase B denaturing conditions with NEB Glycoprotein Denaturing Buffer, containing SDS and DTT, Remove-iT PNGase F yields a higher concentration of 500,000 U/ml.
- If using Remove-iT PNGase F under typical PNGase F denaturing conditions, it is essential to have NP-40 in the reaction mixture as Remove-iT PNGase F is inhibited by SDS. It is not known why this non-ionic detergent counteracts the SDS inhibition.
- Remove-iT PNGase F will not cleave *N*-linked glycans containing core α1-3 Fucose.

(see other side)

- Recommended storage temperature is 4°C, avoid repeat freeze-thaw cycles
- Removal of Remove-iT PNGase F 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. Maley, F. et al. (1989) *Anal. Biochem.* 180, 195–204.
2. Plummer, T. H., Jr. and Tarentino, A.L. (1991) *Glycobiology* 1, 257–263.

Companion Products:

RNase B
#P7817S 250 µg

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