Description: PNGase F PRIME™ is a recombinant glycosidase, cloned from *Flavobacterium meningosepticum*, which catalyzes the cleavage of N-linked oligosaccharides from proteins.

Biological Source: E. coli.

Concentration: 500,000 units/mL [1.5 mg/mL]

Molecular Weight: PNGase F PRIME™ has a molecular weight of approximately 36kDa.

Physical Form: PNGase F PRIME™ is supplied as a liquid in 1X PBS (Phosphate Buffered Saline; 137 mM NaCl, 10mM Phosphate, 2.7 mM KCl, pH7.4) at a concentration of 500,000 units/mL.

Storage Conditions: Store at temperatures ranging from +2° to -20°C. Avoid multiple freeze-thaw cycles.

Unit Definition Assay: Achieves complete deglycosylation of 10 µg of RNase B incubated in 1X PBS with 1 µL of PNGase F PRIME™ for 5-10 minutes at 37°C or room temperature. Separation of reaction products are visualized by SDS-PAGE.

Purity: ≥95% as determined by SDS-PAGE analysis and staining with Coomassie Brilliant Blue™.

Protein Deglycosylation Using Recombinant PNGase F PRIME™

Note: The following protocols are intended as a general guide for protein deglycosylation and may require modification for different glycoprotein substrates.

- Recombinant PNGase F PRIME™ has been optimized for use under non-denaturing conditions, but like many enzyme reactions, activity is substrate dependent and specific conditions should be determined empirically for each target.
- PNGase F PRIME™ is also fully active when used in denaturing reactions and 1 µL PNGase F PRIME™ has been demonstrated to fully deglycosylate 10µg IgG or RNase B within 10 minutes at 50°C.

1. **General Protocol for the deglycosylation of proteins under <u>Denaturing Conditions</u>:**

   Note: Deglycosylation of glycoproteins may be visualized by gel-shift on SDS-PAGE, with the deglycosylated product running faster than the glycosylated substrate.

Materials to Be Supplied By the User:

- 5% SDS
- 1M DTT
- 1X Phosphate Buffered Saline (PBS) (pH 7.4)
- 10% NP-40
- Wet Ice

a) Add up to 50µg of the target glycoprotein in 1X PBS to a final volume of 11µL.
b) Add 1µL 5% SDS.
c) Add 1µL of 1M DTT.
d) Denature sample by heating at 95°C for 10 minutes.
e) Cool sample by placing sample on Ice.

Note: Other buffers can be used if they are within the acceptable pH range for PNGase F PRIME™, pH 6–10.

g) Add 2µL of 10% NP-40.
h) Add 1µL of recombinant PNGase F PRIME™.
i) Incubate at 37°C for 30 minutes.

2. **General Protocol for the deglycosylation of proteins under <u>Non-Denaturing Conditions</u>:**

Note: Deglycosylation under non-denaturing conditions may require increasing both the amount of PNGase F PRIME™ used and the incubation time.

Materials to Be Supplied By the User:

- Phosphate Buffered Saline

a) Add up to 20µg of glycoprotein in 1X PBS (pH 7.4) to a final volume of 18µL.
b) Add 2µL of recombinant PNGase F PRIME™.
c) Incubate at 37°C for 0.5–24 hours.