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: 10^6 Units/mL [2.0 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 >10^6 Units/mL.

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

Unit Definition Assay: Denatured RNase B (10µg) is incubated with PNGase F PRIME™ for 30 minutes at 37°C and then analyzed by SDS-PAGE. Fully glycosylated RNase B migrates at approximately 17kDa. Deglycosylation is assessed by the presence of deglycosylated RNase B with an apparent molecular weight of 13.7 kDa following staining via Coomassie Brilliant Blue™.

High-End Criteria: PNGase F PRIME™ is also designed for use in high-end applications. We therefore include the following rigorous quality release criteria using HPLC/UPLC and Mass Spectrometry Imaging of tissue samples:

- Denatured human IgG (10µg) is incubated with PNGase F PRIME™ for one hour before glycan is labeled with the Waters RapiFluor-MS dye and analyzed by normal phase hydrophilic interaction chromatography (HILIC).

- PNGase F PRIME™ is used for imaging of glycans from tissue sections as described in [Powers et al., PLoS One, 2014, 9(9); p. e106255.] using both a Bruker Daltonics SolariX™ 7T Hybrid FTMS System and a Bruker Daltonics rapifleXTM MALDI Tissuetyper.

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.

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:

- 1X Phosphate Buffered Saline (PBS) (pH 7.4)

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