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

Biological Source: E. coli.

Concentration: 10,000 U/mL.

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

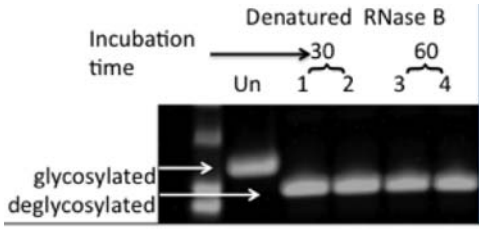

Figure 1. PnGase F Prime™ activity assay. Coomassie stained gel of denatured RNase B after treatment with PNGase F PRIME™ for 30 minutes (lanes 1,2) or 60 minutes (lanes 3,4).

Physical Form: PNGase F PRIME™ is supplied as a liquid in 20mM Tris-HCl (pH 7.5 at 25°C), 50mM NaCl and 5mM EDTA at a concentration of 10,000U/mL.

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

Unit Definition: One unit of PNGase F PRIME™ will catalyze the deglycosylation of 1 nanomole of denatured Ribonuclease B (RNase B) in 30 minutes at 37°C. One unit is equal to 1 IUB milliunit.

This lot passes the following Quality Control specifications:

1. **Activity 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 13.7kDa following staining via Coomassie Brilliant Blue™.
2. **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. Like many enzyme reactions, it is highly dependent on reaction conditions and should be determined empirically for each target.

1. General Protocol for the deglycosylation of proteins under Denaturing Conditions:

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 7.2)
- 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 Non-Denaturing Conditions:

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 7.2) to a final volume of 18µL.
b) Add 2µL of recombinant PNGase F PRIME™.
c) Incubate at 37°C for 0.5–24 hours.