**Description:** PNGase F PRIME-LY<sup>M</sup> is a lyophilized recombinant glycosidase, cloned from *Flavobacterium meningosepticum*, which catalyzes the cleavage of N-linked oligosaccharides from proteins.

**Biological Source:** *E. coli.* 

**Concentration:** Each standard vial of lyophilized format enzyme contains 100  $\mu$ g of PRIME<sup>TM</sup> endoglycosidase lyophilized from 20mM Tris-HCl, 50mM NaCl, pH 7.5. The final concentration of the enzyme is ultimately determined by the amount of dH<sub>2</sub>O used in the reconstitution process.

**Molecular Weight:** PNGase F PRIME-LY<sup>™</sup> has a molecular weight of approximately 36kDa.

**Physical Form:** PNGase F PRIME-LY<sup>™</sup> is supplied as a dry white powder.

**Storage Conditions:** PNGase F PRIME-LY<sup>TM</sup> is stable at RT and can be stored at RT upon receipt. PNGase F PRIME-LY<sup>TM</sup> is reconstituted by adding dH<sub>2</sub>O and vortexed. After reconstitution, the PNGase F PRIME-LY<sup>TM</sup> is stable for 1 month at +4°C but should be kept at -20°C for long term storage. After reconstitution, avoid multiple freeze-thaw cycles.

**Unit Definition Assay:** Denatured RNase B (10µg) is incubated with reconstituted PNGase F PRIME-LY<sup>TM</sup> 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<sup>TM</sup>.

**High-End Criteria**: Reconstituted PNGase F PRIME-LY<sup>™</sup> 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 reconstituted PNGase F PRIME-LY<sup>™</sup> for one hour before glycan is labeled with the Waters RapiFluor-MS dye and analyzed by normal phase hydrophilic interaction chromatography (HILIC).
- Reconstituted PNGase F PRIME-LY<sup>™</sup> is used for imaging of glycans from tissue sections as described in [*Powers et al., PLoS One. 2014, 9(9): e106255.*] using both a Bruker Daltonics SolariX<sup>™</sup> 7T Hybrid FTMS System and a Bruker Daltonics rapifleXTM MALDI Tissuetyper.

**Purity:**  $\geq$  95% as determined by SDS-PAGE analysis and staining with Coomassie Brilliant Blue<sup>M</sup>.

## Protein Deglycosylation Using Reconstituted Recombinant PNGase F PRIME-LY™

**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-LY<sup>™</sup> has been optimized for use under nondenaturing conditions, but like many enzyme reactions, activity is substrate dependent and specific conditions should be determined empirically for each target.
- Reconstituted PNGase F PRIME-LY<sup>™</sup> 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  $50\mu g$  of the target glycoprotein in 1X PBS to a final volume of  $11\mu L$ .
- 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 reconstituted PNGase F PRIME-LY<sup>™</sup>, pH 6–10.

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

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

**Note:** Deglycosylation under non-denaturing conditions may require increasing both the amount of reconstituted PNGase F PRIME-LY<sup>™</sup> used and the incubation time.

## Materials to Be Supplied By the User:

- 1X Phosphate Buffered Saline (PBS) (pH 7.4)
- a) Add up to  $20\mu g$  of glycoprotein in 1X PBS to a final volume of  $18\mu L$ .
- b) Add 2µL of reconstituted recombinant PNGase F PRIME-LY™.
- c) Incubate at 37°C for 0.5–24 hours.