Description: PNGase F PRIME-LY™ ULTRA is a lyophilized recombinant glycosidase optimized for high-resolution imaging of small structures or cells. This high-performance enzyme is 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 µg of PRIME-LY™ ULTRA 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_2O used in the reconstitution process.

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

Physical Form: PNGase F PRIME-LY™ ULTRA is supplied as a dry white powder.

Storage Conditions: PNGase F PRIME-LY™ ULTRA is stable at RT and can be stored at RT upon receipt. PNGase F PRIME-LY™ ULTRA is reconstituted by adding dH_2O and vortexed. After reconstitution, the PNGase F PRIME-LY™ ULTRA 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™ ULTRA 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: Reconstituted PNGase F PRIME-LY™ ULTRA is 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™ ULTRA 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™ ULTRA 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™ 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 Reconstituted Recombinant PNGase F PRIME-LY™ ULTRA

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™ ULTRA 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.
- Reconstituted PNGase F PRIME-LY™ ULTRA 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 reconstituted PNGase F PRIME-LY™ ULTRA, pH 6–10.

g) Add 2µL of 10% NP-40.
h) Add 1µL of reconstituted recombinant PNGase F PRIME-LY™ ULTRA.
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 reconstituted PNGase F PRIME-LY™ ULTRA 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 reconstituted recombinant PNGase F PRIME-LY™ ULTRA.
c) Incubate at 37°C for 0.5–24 hours.