ATAGENIX LABORATORIES

Catalog Number:ATE00005

PNGase F

Product overview

Product Name	PNGase F
Catalog No.	ATE00005
Expression host	E.coli
Restriction sites	PNGase F works by cleaving between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides from N-linked glycoproteins and glycopeptides. This results in a deaminated protein or peptide and a free glycan.
MW	36kDa
Purity	>95% as determined by SDS-PAGE quantitative densitometry by Coomassie Blue Staining.
Concentration	1800000 U/mg
Unit Definition	One unit of PNGase F will catalyze more than 95% of the deglycosylation of 10μg of denatured Ribonuclease B(RNase B) in 10 ul reaction system in one hour at 37° C.
Digestion reaction condition	At 37 ℃, the enzyme digestion reaction 1h, every 1ug enzyme can digest 10ug substrate. (The mass ratio of enzyme to substrate is determined by our company based on the degree of glycosylation of the substrate used, for reference only)

Product performance

Form	Liquid
Buffer	20 mM Tris-HCl pH 7.5, 50 mM NaCl, 5 mM EDTA,50%Glycerol
Storage	Store at -20 °C for 12 months from the date of receipt.

Standard Operating Procedure

1: Protein Deglycosylation Using Denaturing Conditions for SDS-PAGE a.Add up to 50μg of the target glycoprotein in water (or a compatible buffer at a low ionic strength) to a final volume of 12μl.

b. Add 1μl of 5% SDS.

c. Add 1μl of 1M DTT.

d. Denature sample by heating at 95°C for 5 minutes.

e. Cool sample for 5 minutes at room temperature.

f. Add 2µl of 0.5M sodium phosphate buffer (pH 7.5). Note: Other buffers can be used if they are within the acceptable pH range for PNGase F (pH 6–10).

g. Add 2µl of 10% NP-40. Note: Triton X-100 may be substituted for NP-40. H.Add 2µl of recombinant PNGase F. I.Incubate at 37°C for 1–3 hours.

2: Protein Deglycosylation Using Non-Denaturing Conditions for Mass Spectrometry

a. Add up to 20µg of glycoprotein in 50mM ammonium bicarbonate (pH 7.8) to a final volume of 18µl.

b. Add 2µl of recombinant PNGase F.

c. Incubate at 37°C for 2–18 hours.