PNGase F

Summary

Catalog No.	YXX04901
Form	Liquid
Storage buffer	20mM Tris-HCl, pH 7.5, 50mM NaCl, 5mM Na2EDTA, 50% glycerol
Concentration	50,000 units/ml
Purity	>95% as determined by SDS-PAGE.
Applications	Removal of high mannose N-glycans from glycoproteins
Target	PNGase F
Biological activity	One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 µg of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl.
Endotoxin level	Please contact with the lab for this information.
Expression system	E. coli
Protein length	PNGase F is cloned from Elizabethkingia miricola and expressed in E.coli.
Nature	Recombinant
Predicted molecular weight	37.08 kDa
Stability and Storage	Use a manual defrost freezer and avoid repeated freeze thaw cycles.Store at 2 to 8 °C for one week .Store at -20 to -80 °C for twelve months from the date of receipt.

Experimental Procedure

Standard Operating Procedure Denaturing Reaction Conditions: 1. Combine 1-20 µg of glycoprotein, 1 µl of Glycoprotein Denaturing Buffer (10X) and H₂O (if necessary) to make a 10 µl total reaction volume. 2. Denature glycoprotein by heating reaction at 100°C for 10 minutes. 3. Chill denatured glycoprotein on ice and centrifuge 10 seconds. 4. Make a total reaction volume of 20 µl by adding 2 µl GlycoBuffer 2 (10X), 2 µl 10% NP-40 and 6 µl H2O. 5. Add 1 µl PNGase F, mix gently. 6. Incubate reaction at 37°C for 1 hour. Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels. Note: To deglycosylate different glycoprotein, longer incubation time may be required.   Non-Denaturing Reaction Conditions: 1.Combine 1-20 µg of glycoprotein, 2 µl of GlycoBuffer 2 (10X) and H2O (if necessary) to make a 20 µl total reaction volume. 2.Add 2-5 µl PNGase F, mix gently. 3.Incubate reaction at 37°C for 4 - 24 hours. Note: To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required. Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels.   1X Glycoprotein Denaturing Buffer
 0.5% SDS
 40 mM DTT

 1X NP-40
 1% NP-40 in MilliQ-H2O

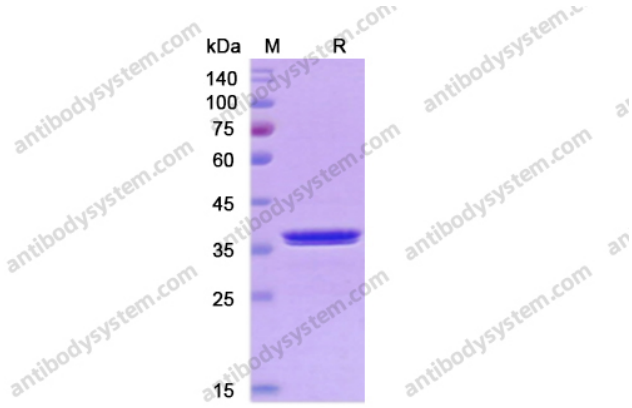
 7.5

Species	Elizabethkingia miricola
Shipping	In general, proteins are provided as lyophilized powder/frozen liquid. They are shipped out with dry ice/blue ice unless customers require otherwise.
Note	For research use only.

Description

Peptide -N-Glycosidase F, also known as PNGase F, is an amidase that cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides from N-linked glycoproteins

Data Image



SDS-PAGE

SDS PAGE for recombinant Elizabethkingia miricola PNGase F

Bioactivity

The deglycosylation of protein detect by SDS-PAGE

Experiment Example

Lane1 : Before cleavage
Lane2 : After cleavage
The control protein was cleaved by PNGase F at 37°C for 1 h under denaturing conditions.