KRISHZYME™ PNGase-F

REF : KPGF-002

Ver 1.0

RIUO

RIUO	**For Research & Industrial Use Only**	**REF**	**Catalog Number**
	Store At	**LOT**	**Batch Code**
	Manufactured By		**Biological Risk**
	Expiry Date		**Consult Operating Instructions**

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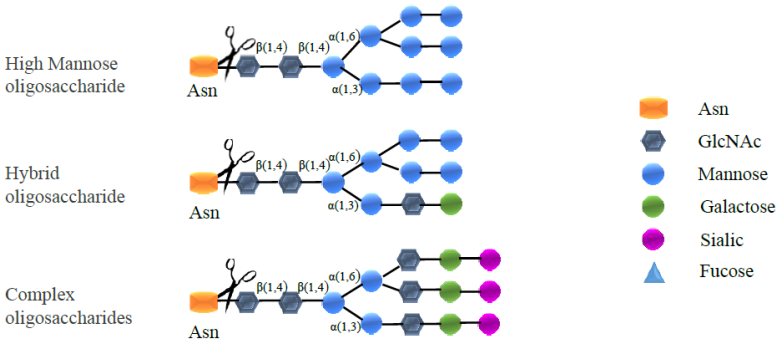
Product Description:
Peptide N-glycosidase F, commonly referred to as PNGase F, is an amidase of the peptide-N4-(N-acetyl-beta-glucosaminyl) asparagine amidase class. PNGase F is the most effective enzymatic method for removing almost all N-linked oligosaccharides from glycoproteins.

KRISHZYME™ PNGase F is a recombinant glycosidase cloned from *Flavobacterium meningsepticum* and overexpressed in *E. coli*. PNGase F has a molecular weight of 36kDa. It works by cleaving between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides unless α(1-3) core fucosylated from N-linked glycoproteins and glycopeptides. This results in a deaminated protein or peptide and a free glycan. Phosphate, sulfate and sialic acid groups attached to the oligosaccharide do not affect cleavage.



KRISHZYME™ PNGase F cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides.

Product Size：

Catalog number	Pack Size	Concentration
KPGF-002-A	15,000 U / 30 ul	500,000 U /ml
KPGF-002-B	5x15,000 U / 30 ul	

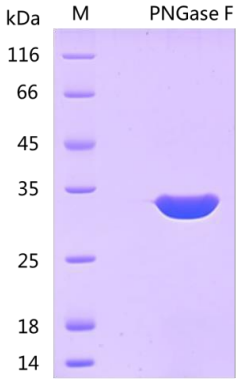
Physical Form:
KRISHZYME™ PNGase F is supplied as a liquid in 20mM Tris-HCl (pH 7.5 at 25°C), 50mM NaCl and 5mM EDTA at a concentration of 500,000 U/ml.

Reagents Supplied:
The following reagents are supplied with this product:

Composition	Formula	Concentration
Denaturing Buffer	5%SDS, 0.4M DTT	10X
Assay Buffer 2	0.5M Sodium Phosphate (pH7.5 at 25°C)	10X
NP-40 Solution		10%

Product Source:
Recombinant gene cloned from *Flavobacterium meningsepticum* and expressed in *E. coli*.

Product Quality:
≥95% purity, as determined by SDS-PAGE. No other exoglycosidase, endoglycosidase, and protease activity were contaminated.



≥95% purity, as determined by SDS-PAGE.

Unit Definition:
One unit of PNGase F is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 ug of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 ul.

Reagent companies differ in how a unit of enzyme is defined. This chart can be used to help determine how a unit of enzyme from one company compares to a unit of enzyme from KRISHGEN.

Enzyme	Company	Selling Conc (U/ml)	Units / Vial	Units / Vial	Krishzyme™ Assay (U/ml)	Krishzyme™ Assay (Units/Vial)	ul Conversion (1 Krishzyme™ ul = X Company ul)
PNGase F	Krishzyme™ KPGF-001/KPGF-002	5,00,000	15,000	30	5,00,000	15,000	1
	NEB (P0708)	5,00,000	15,000	30	5,00,000	15,000	1
	Prozyme (GKE-5006A)	2.5	0.1	40	1,50,000	6,000	3.3
	Prozyme (GKE-5020B, ultra)	10	0.4	40	5,00,000	20,000	1
	QA Bio (E- PNG01)	5	0.3	60	2,00,000	12,000	2.5
	Sigma (P7367)	500	50	50	90,000	4,500	5.5

Storage Temperature:
4°C

Characteristic :
- Recombinant enzyme
- Stored in 50% glycerol.
- ≥95% purity, as determined by SDS-PAGE
- Optimal activity and stability for up to 12 months
- Can be used under native or denaturing conditions
- Optimized for deglycosylation of glycoproteins; leaves N-glycan core oligosaccharides intact and suitable for further analysis

Applications:

- Characterizing whether the protein is glycosylated
- Release of intact N-linked glycans from glycopeptides and glycoproteins
- Structure-function studies of N-glycosylated glycoproteins
- Preparation of deglycosylated proteins for molecular weight estimation or crystallography studies

Suggestions for Use:

Denaturing Reaction Conditions:

1) Combine 10 - 100 ug of glycoprotein, 1 ul of **10X Denaturing Buffer** and **H_2O** (if necessary) to make a 10 ul 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 ul by adding **2 ul** of **10X Assay Buffer 2**, **2 ul** of **10% NP- 40 Solution** and **6 ul H_2O**;
5) Add 1 ul PNGase F, mix gently;
6) Incubate reaction at 37°C for 1-3 hours.
7) Use downstream for analysis.

Non-Denaturing Reaction Conditions:

1) Combine 10 - 100 ug of glycoprotein, **2 ul of (10X) Assay Buffer 2**, **2-5 ul PNGase F** and **H_2O** (if necessary) to make a 20 ul total reaction volume, mix gently;
2) Incubate reaction at 37°C for 4-24 hours;
3) Use downstream for analysis.

Notes :

- Since KGPF-002 contains 50% glycerol, we recommend limiting PNGase F to 1/10 (or less) of the total reaction volume to keep the final glycerol concentration equal to (or less than) 5%.
- When deglycosylating a native glycoprotein, it is recommended that an aliquot of the glycoprotein is subjected to the denaturing protocol to provide a positive control for the fully deglycosylated protein. The non-denatured reaction can then be compared to the denatured reaction to determine the extent of reaction completion;
- To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required;
- The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels;
- Since PNGase F, Recombinant activity is inhibited by SDS, it is essential to have NP- 40 in the reaction mixture. It is not known why this non-ionic detergent counteracts the SDS inhibition at the present time;
- PNGase F, Recombinant will not cleave N-linked glycans containing core α1-3 Fucose;
- Recommended Storage Temperature is -20°C.

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