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KRISHZYME™ Swift™ PNGase-F, lyophilized

REF : KPGF-001

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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REF **KPGF-001**

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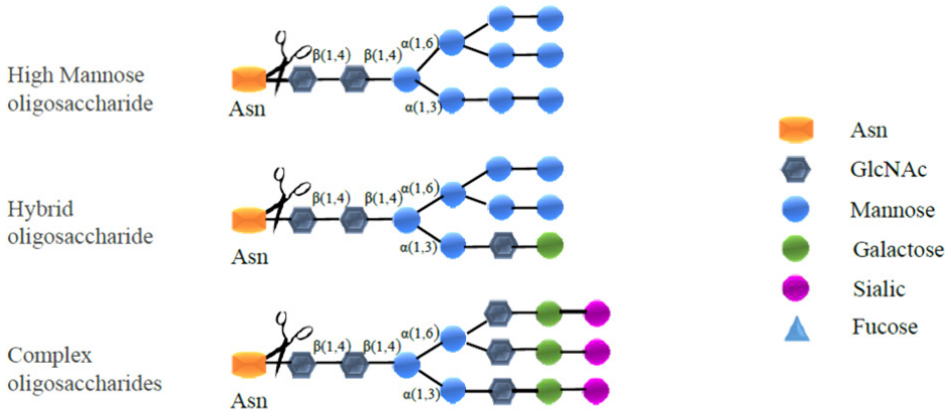
Product Description:

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™ Swift 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-001-A	50,000U / 50 ul	1,000,000 U /ml
KPGF-001-B	2 x 50,000U / 50 ul	

Physical Form:

KRISHZYME™ Swift™ PNGase F is supplied as a vial of enzyme containing 100 ug of PNGase-F lyophilized from 20mM Tris-HCl, 50mM NaCl, pH 7.5. Resuspend in 50 ul of double distilled water to get a concentration of 1,000 U/ul or 50,000 U/vial.

Reagents Supplied:

The following reagents are supplied with this product:

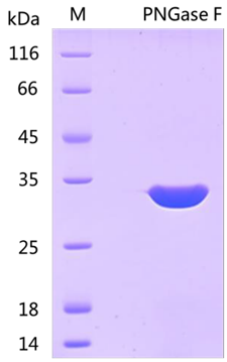
Composition	Formula	Concentration
Denaturing Buffer	5%SDS, 0.4M DTT	10X
Assay Buffer 2	0.5M Sodium Phosphate (pH7.5 at 25°C)	10X
NP-40 Solution		10%

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.

Storage Temperature:
4°C

Characteristic:

- Recombinant enzyme
- Glycerol-free for optimal performance in HPLC and mass spectrometry analysis
- ≥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
- Determining the location of glycosylation on the protein
- Characterizing the glycan structure
- Protein trafficking
- 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 10×Denaturing Buffer and H2O (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 10×Assay Buffer 2, 2 ul 10% NP- 40 Solution and 6 ul H2O;
5) Add 1 ul PNGase F, mix gently;
6) Incubate reaction at 37°C for 1-3 hours.
7) Analyze by method of choice.

Non-Denaturing Reaction Conditions:

1) Combine 10 - 100 ug of glycoprotein, 2 ul of (10X) Assay Buffer 2, 2-5 ul PNGase F (Glycerol-free) and H2O (if necessary) to make a 20 ul total reaction volume, mix gently;
2) Incubate reaction at 37°C for 4-24 hours;
3) Analyze by method of choice.

Notes :

- 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 (Glycerol-free), 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 (Glycerol-free), Recombinant will not cleave N-linked glycans containing core α1-3 Fucose;
- Recommended storage temperature is 4°C, avoid repeat freeze-thaw cycles.

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