KRISHZYME™ PROTEIN DEGLYCOSYLATION-NO2 KIT

REF : KPGF-300

Ver 1.0

RIUO

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

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Product Description:
KRISHZYME™ Protein Deglycosylation-NO2 Kit (for N-linked & Complex O-linked Glycans) contains 1 vial each of PNGase F (Glycerol-free) (Krishzyme™ KPGF-001), O-Glycosidase (Krishzyme™ KPGF-004) and α2-3,6,8,9 Neuraminidase (Krishzyme™ KPGF-005), β1-4 Galactosidase (Krishzyme™ KPGF-006) and β-N-Acetylhexosaminisase (Krishzyme™ KPGF-007) which can be used simultaneously for the removal of all N-linked Glycans and simple O-linked Glycans (including polysialylated) from glycoproteins.

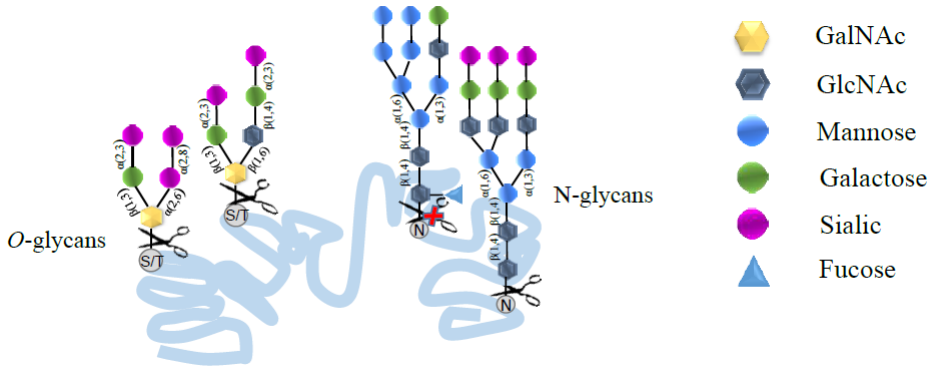
Krishzyme™ PNGase F (Glycerol-free) cleaves all N-linked complex, hybrid or high mannose oligosaccharides unless α(1,3) core fucosylated (asparagine must be peptide-bonded at both termini).

Krishzyme™ O-Glycosidase, also known as Endo-α-N-Acetylgalactosaminidase, catalyzes the removal of Core 1 and Core 3 O-linked disaccharides from glycoproteins. Unfortunately there is no such "universal" endoglycosidase for O-glycans, these have to be trimmed down to Gal-GalNAc-O-S/T or GlcNAc-β(1-3)-GalNAc-O-S/T before O-glycanase can remove the Core structure. Modification of this core with e.g. sialic acid will block O-glycanase.

Krishzyme™ α2-3,6,8,9 Neuraminidase is included in the kit and can remove α(2,3)-, α(2,6)-, α(2,8)-, and α(2,9)-linked N-acetylneuraminic Acid from glycoproteins and oligosaccharides.

β1-4 Galactosidase and β-N-Acetylhexosaminisase can assist with removal of e.g. core 2 structures (GlcNAc-Gal- GalNAc-S/T).

One set of this Kit includes 15,000 units of PNGase F (Glycerol-free); 1,200,000 units of O-Glycosidase and 0.6 units of α2-3,6,8,9 Neuraminidase, 0.06 units of β1-4 Galactosidase and 150 units of β-N-Acetylhexosaminisase.



KRISHZYME™ Protein Deglycosylation-NO2 Kit (for N-linked & Complex O-linked Glycans) can be used simultaneously for the removal of all N-linked Glycans and complex O-linked Glycans (including polysialylated) from glycoproteins.

Product Size :

Cat No	Pack Size	Concentration
KPGF-300	PNGase F(Glycerol-free)	15,000 U / 30 ul
	O-Glycosidase	1,200,000 U / 30 ul
	α2-3,6,8,9 Neuraminidase	0.6 U / 30 ul
	β1-4 Galactosidase	0.06 U / 30 ul
	β-N-Acetylhexosaminisase	150 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.

KRISHZYME™ O-Glycosidase is supplied as a liquid in 20mM Tris-HCl (pH 7.5 at 25°C), 50mM NaCl and 1mM EDTA.

KRISHZYME™ α2-3,6,8,9 Neuraminidase is supplied as a liquid in 20mM Tris-HCl (pH 7.5 at 25°C), 50mM NaCl and 1mM EDTA.

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:
Krishzyme™ PNGase-F (Glycerol free) is cloned from *Elizabethkingia meningoseptica* and expressed in *E.coli*. O-Glycosidase is cloned from *Enterococcus faecalis* and expressed in *E.coli*. α2-3,6,8,9 Neuraminidase is cloned from *Clostridium perfringens* and overexpressed in *E.coli*. β1-4 Galactosidase is cloned from Streptococcus pneumoniae and expressed in *E.coli*. β-N-Acetylhexosaminidase is cloned from Streptomyces plicatus and overexpressed in *E.coli*.

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

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.

One Unit of O-Glycosidase is defined as the amount of enzyme required to remove 0.68 nmol of O-linked disaccharide from 5mg of neuroaminidase digested, non-denatured fetuin in one hour at 37ºC in a total reaction of 100 ul.

One Unit of α2-3,6,8,9 Neuraminidase is defined as the amount of enzyme required to catalyze the release of 1 umole of p-nitrophenol from p-nitrophenyl-α-D-N-acetylneuroaminic acid per minute at 37ºC, pH5.5.

One unit of β1-4 Galactosidase is defined as the amount of enzyme required to hydrolyze 1 µmole oNP-β-D-galactopyranoside per min at pH 6.0 and 37℃.

One unit of β-N-Acetylhexosaminidase is defined as the amount of enzyme required to catalyze the release of 1 µmole of p-nitrophenol from p-nitrophenol-N-acetyl-β-D-glucosaminide per minute at 37°C, pH 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, 3 ul of **10X Denaturing Buffer** and H2O (if necessary) to make a 30 ul total reaction volume.
2) Denature glycoprotein by heating reaction at 100°C for 10 minutes.
3) Make a total reaction volume of 50 ul by adding **5 ul of 10X Assay Buffer 2**, **5 µl of 10% NP40 Solution**, **1-2 µl of PNGase-F (Glycerol-free)**, **1-2 µl of O-Glycosidase**, **1-2 µl of α2-3,6,8,9 Neuraminidase**, **1-2 µl of β1-4Galactosidase**, **1-2 µl of β-N-Acetylhexosaminidase** and H_2O, mix gently.
4) Incubate reaction at 37°C for 1-5 hours.

Non-Denaturing Reaction Conditions:

1) Combine 10–100 ug of glycoprotein, **5 ul of 10X Assay Buffer 2**, **1-2 ul of PNGase-F (Glycerol-free)**, **1-2 ul of O-Glycosidase**, **1-2 ul of α2-3,6,8,9 Neuraminidase**, **1-2 ul of β1- 4 Galactosidase**, **1-2 ul of β-N-Acetylhexosaminidase** and H_2O to make a 50 ul total reaction volume, mix gently.
2) Incubate reaction at 37°C for 16 hours.

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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