KRISHZYME™ PROTEIN DEGLYCOSYLATION-O KIT

REF : KPGF-100

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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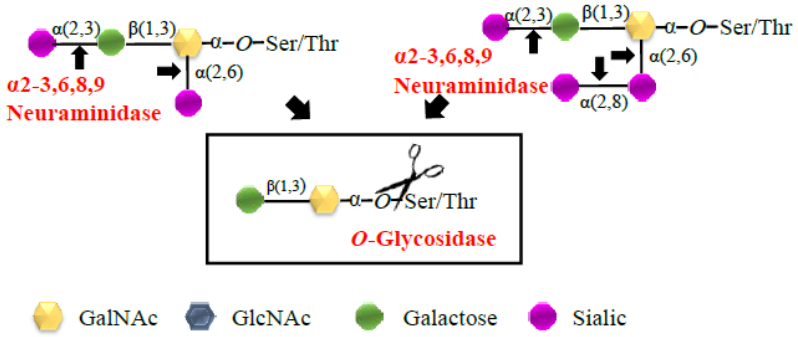
Product Description:
KRISHZYME Protein Deglycosylation-O Kit (for Simple O-linked Glycans) contains O-Glycosidase (Krishzyme™ KPGF-004) and α2-3,6,8,9 Neuraminidase (Krishzyme™ KPGF-005), which can be used simultaneously for the removal of terminal sialic acid residues and core 1 and core 3 O-glycans. One set of this Kit includes 1,200,000 units of O-Glycosidase and 0.6 units of α2-3,6,8,9 Neuraminidase.

KRISHZYME™ O-Glycosidase, also known as Endo-α-N-Acetylgalactosaminidase, catalyzes the removal of Core 1 and Core 3 O-linked disaccharides from glycoproteins. KRISHZYME™ α2-3,6,8,9 Neuraminidase is the common name for Acetyl-neuraminyl hydrolase (Sialidase). This Neuraminidase catalyzes the hydrolysis of α2-3, α2-6, α2-8and α2-9linked N-acetyl-neuraminic acid residues from glycoproteins and oligosaccharides.



KRISHZYME™ Protein Deglycosylation-O Kit (for Simple O-linked Glycans) can be used simultaneously for the removal of terminal sialic acid residues and core 1 and core 3 O-glycans. One set of the Kit includes 1,200,000 units of O-Glycosidase and 0.6 units of α2-3,6,8,9 Neuraminidase.

Product Size：

Cat No	Pack Size	Concentration
KPGF-100	O-Glycosidase α2-3,6,8,9 Neuraminidase	1,200,000 U / 30 ul 0.6 U / 30 ul

Physical Form:
KRISHZYME™ O-Glycosidase and α2-3,6,8,9 Neuraminidase are supplied as a liquid in 20mM Tris-HCl (pH 7.5 at 25°C), 50mM NaCl , 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:
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*.

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

Unit Definition:
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.

Storage Temperature:
-20°C

Characteristic :
- Recombinant enzyme with no detectable endoglycosidase or other exoglycosidases contaminating activities
- ≥95% purity, as determined by SDS-PAGE
- Optimal activity and stability for up to 24months
- Glycerol-free for optimal performance in HPLC and mass spectrometry analysis
- Effective for the hydrolysis of sialylated O-linked oligosaccharides

Applications:
- Structural analysis of oligosaccharides
- Glycoprotein deglycosylation
- Removing heterogeneity from glycoproteins
- Characterization and Quality Control of Therapeutic Glycoproteins

Suggestions for Use:

Denaturing Reaction Conditions:
1. Combine 10–100 ug of glycoprotein, 1 ul of 10X 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. Make a total reaction volume of 20 ul by adding 2 ul 10X Assay Buffer 2, 2 ul 10%NP-40 Solution, 1-2 ul of α2-3,6,8,9 Neuraminidase, H2O and 1-2 ul of O-Glycosidase, mix gently.
4. Incubate reaction at 37°C for 1-4 hours.

Non-Denaturing Reaction Conditions:
1. Combine 10–100 ug of glycoprotein, 2 ul of 10XAssay Buffer 2, 1-2 ul of α2-3,6,8,9 Neuraminidase, H2O and 1-2 ul O-Glycosidase to make a 20 ul total reaction volume, mix gently.
2. Incubate reaction at 37°C for 1-4 hours.

Notes :
- Since O-Glycosidase is inhibited by SDS, it is essential to have NP-40 in the reaction mixture.
- To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.

References:
Characterization of glycoproteins and their associated oligosaccharides through the use of endoglycosidases. ... F Maley, RB Trimble, AL Tarentino… - Analytical biochemistry, 1989 - Elsevier

Deglycosylation of asparagine-linked glycans by peptide: N-glycosidase F. ... AL Tarentino, CM Gomez, TH Plummer Jr - Biochemistry, 1985 - ACS Publications/

Demonstration of peptide: N-glycosidase F activity in endo-beta-N-acetylglucosaminidase F preparations. ... TH Plummer, JH Elder, S Alexander, AW Phelan… - Journal of Biological …, 1984 - ASBMB

Glycosylation of Pichia pastoris -derived proteins. ... RK Bretthauer, FJ Castellino - Biotechnology and applied …, 1999 - Wiley Online Library.

Pharmacological chaperones rescue cell-surface expression and function of misfolded V2 vasopressin receptor mutants. ... JP Morello, A Salahpour, A Laperrière… - The Journal of …, 2000 - Am Soc Clin Investig.

A monolithic PNGase F enzyme microreactor enabling glycan mass mapping of glycoproteins by mass spectrometry. ... AK Palm, MV Novotny - … An International Journal Devoted to the …, 2005 - Wiley Online Library.

Protein-protein interaction and not glycosylation determines the binding selectivity of heterodimers between the calcitonin receptor-like receptor and the … S Hilairet, SM Foord, FH Marshall, M Bouvier - Journal of Biological …, 2001 - ASBMB

Purification and Structure-Function Analysis of Native, PNGase F-Treated, and Endo-. beta.-galactosidase-Treated CHIP28 Water Channels. AN Van Hoek, MC Wiener, JM Verbavatz, D Brown… - Biochemistry, 1995 - ACS Publications

Isolation and characterization of CD47 glycoprotein: a multispanning membrane protein which is the same as integrin-associated protein (IAP) and the ovarian tumour … WJ Mawby, CH Holmes, DJ Anstee, FA Spring… - Biochemical …, 1994 - biochemj.org

DC-SIGN and L-SIGN are high affinity binding receptors for hepatitis C virus glycoprotein E2
…, S Foung, A Amara, C Houles, F Fieschi… - Journal of Biological …, 2003 - ASBMB

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