# KRISHZYME® Endo-alpha-N-Acetylgalactosaminidase (O-Glycosidase)

**REF** : KPGF-004

Ver 1.0

**RIUO**

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| **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-004**

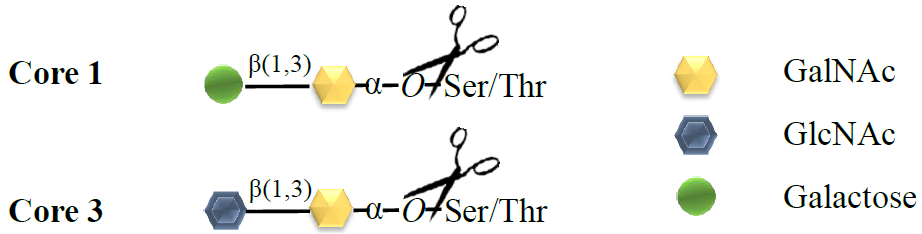
**Krishgen Biosystems Private Limited**
For US/Europe Customers: toll free +1(888)-970-0827 | tel +1(562)-568-5005
For Asia/India Customers: +91(22)-49198700
Email: sales1@krishgen.com | http://www.krishgen.biz

**Product Description:**

KRISHZYME® Endo-alpha-N-Acetylgalactosaminidase (O-Glycosidase), also known as Endo-α-N-Acetylgalactosaminidase, specifically catalyzes the removal of Core 1 and Core 3 O-linked disaccharides from glycoproteins.

KRISHZYME® Endo-alpha-N-Acetylgalactosaminidase (O-Glycosidase) is a recombinant glycosidase cloned from Enterococcus faecalis and expressed in E. coli.

KRISHZYME® Endo-alpha-N-Acetylgalactosaminidase (O-Glycosidase) has a molecular weight of 147kDa. This product is a highly specific enzyme, which hydrolyzes the N-acetylgalactosamine glycosidic linkage, liberating the core disaccharide from the serine or threonine residue that is either unsubstituted or present in glycoproteins or glycopeptides.

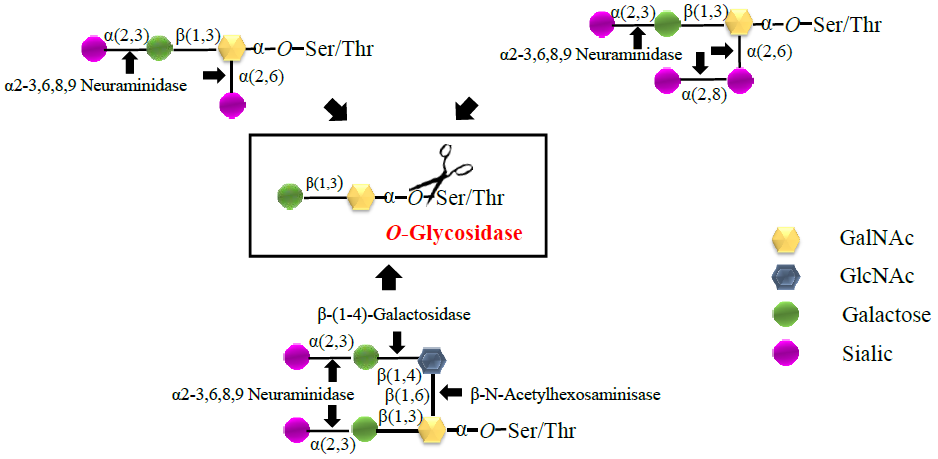



*KRISHZYME® Endo-alpha-N-Acetylgalactosaminidase (O-Glycosidase) catalyzes the removal of Core 1 and Core 3 O-linked disaccharides from glycoproteins.*

The enzyme is specific for a-GalNAc linkages, but it has no apparent preference for serine over threonine-linked residues. Any modification of the core structure can block the action of O- glycosidase. If the O-glycan structure is larger than the core structure, for example substituted with N-acetylglucosamine, N-acetylgalactosamine, sialic acid, or fucose, O-glycosidase will not cleave the GalNAc to Ser/Thr linkage.

In such cases the additional monosaccharides must be sequentially hydrolyzed by a series of exoglycosidases, until only the Gal-β(1-3)-GalNAc- or GlcNAc-β(1-3)- GalNAc- core remains. O-Glycosidase can then remove the core structure intact with no modification of the serine or threonine residue.

Usually it is necessary to treat glycoproteins concomitantly with Neuraminidase and O-Glycosidase. Neuraminic Acid residues must be removed in order to allow O-Glycosidase to cleave the O-linked disaccharides. A general Neuraminidase (KPGF-005) or the Protein Deglycosylation-O Kit (for Simple O-Linked Glycans) (KPGF-100) is recommended.




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**Product Size :**

| Cat No | Pack Size | Concentration |
|---|---|---|
| KPGF-004-A | 0.07 U / 50 ul | 1.4 U /ml |
| KPGF-004-B | 0.28 U / 200 ul | |

**Physical Form:**

KRISHZYME® Endo-alpha-N-Acetylgalactosaminidase (O-Glycosidase) is supplied as a liquid in 20mM Tris-HCl (pH 7.5 at 25°C), 50mM NaCl and 1mM EDTA at a concentration of 1.4 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 Enterococcus faecalis and expressed 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 5 mg of neuraminidase digested, non-denatured fetuin in 1 hour at 37°C in a total reaction volume of 100 ul.

**Storage Temperature:**
Store at -20°C.

**Characteristic :**

- 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 24months
- Can be used under native or denaturing conditions
- Can be used in conjunction with other exoglycosidases to remove more complex O-glycans
- Recombinant enzyme with no detectable exoglycosidase or other endoglycosidase contaminating activities

**Applications:**

- Detection of O-glycosylation in proteins
- Epitope and binding site analysis
- Bioactivity in O-glycans

- Analysis of biosynthesis of glycoproteins
- Structural analysis of O-glycan
- Glycoprotein deglycosylation

**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 of 10X Assay Buffer 2, 2 ul of 10% NP-40 Solution, 1-2 ul of α2-3,6,8,9 Neuraminidase, H2O and 1-2 ul 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 10X Assay 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.

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