

GlySERIAS™

Immobilized

FOR RESEARCH
USE ONLY
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STORE AT
+4-8°C



SmartEnzymes™



GENOVIS

INSTRUCTIONS FOR PRODUCTS

GlySERIAS Immobilized Microspin 5×0.2mg

Process 5×0.2 mg fusion protein (A0-GS6-010)

GlySERIAS Immobilized Microspin 10×0.2mg

Process 10×0.2 mg fusion protein (A0-GS6-020)

Quick Guide

1 Equilibration

- Equilibrate the column with $3 \times 300 \mu\text{l}$ of digestion buffer. Centrifuge at $200 \times g$ for 1 min.



2 Digestion

- Add the fusion protein to the GlySERIAS Immobilized column and cap the column. Incubate with end-over-end mixing at room temperature for 1 h to overnight.



3 Collection

- Centrifuge at $1000 \times g$ for 1 min to collect the digested fusion protein.
- For maximum recovery, add $100 \mu\text{l}$ of digestion buffer, resuspend the media and centrifuge at $1000 \times g$ for 1 min.



PRODUCT DESCRIPTION

GlySERIAS Immobilized is a resin with the GlySERIAS enzyme covalently coupled to agarose beads for digestion of flexible linkers in fusion proteins. The enzyme is cloned from Phage K and is recombinantly expressed in *E. coli*.

GlySERIAS digests flexible linkers such as $(\text{Gly}_4\text{Ser})_n$, Gly_xSer_y or exclusively polyglycine linkers in engineered fusion proteins containing two or more protein or peptide domains.

The repetitive design of the linker will lead to several simultaneous digestion sites and separation of the previously linked proteins.

The fusion protein is incubated with the GlySERIAS Immobilized resin in a spin column for 1 h to overnight using non-denaturing reaction conditions.

The digested protein is then easily collected by a centrifugation step. The activity of GlySERIAS can be inhibited or slowed down by steric hindrance or the nature of the linker. In such cases, longer incubation times may be required.

Content and Storage

Each GlySERIAS Immobilized Microspin column contains sufficient material to digest 0.2 mg fusion protein. The resin is supplied in 20% EtOH with no preservatives added.

GlySERIAS Immobilized Microspin columns are shipped cold and should be stored at +4-8°C upon arrival. **Do not freeze the product!**

GlySERIAS Immobilized Microspin is for R&D use only.

DETAILED PROTOCOL

Use lids and bottom caps during the incubation.

Before centrifugation, remove the bottom cap and loosen the lid (do not remove the lid).

Equipment Required

- Centrifuge for microcentrifuge tubes
- Equipment enabling end-over-end mixing

Additional Materials Required

- Digestion buffer: TBS, pH 7.6 or PBS, pH 7.4.
- Collection tubes: Microcentrifuge tubes (1.5-2 ml)

Sample Preparation

Prepare the fusion protein in 100-300 μ l of digestion buffer¹ per column. Recommended amount of glycoprotein is 0.2 mg per column.

1 Equilibration

- Break off the bottom cap of the column (save the cap) and place the column in a collection tube. Loosen the lid.
- Centrifuge at $200 \times g$ for 1 min to remove the storage solution.
- Equilibrate the column by adding 300 μ l of digestion buffer and centrifuge at $200 \times g$ for 1 min.
- Perform the equilibration steps above three times.
- Seal the spin column with the bottom cap.

2 Digestion

- Add the fusion protein to the column (0.2 mg in 100-300 μ l of digestion buffer).
- Seal the column with the top lid.
- Fully suspend the media. Mix it by inversion and make sure there is a flow in the column.
- Incubate the column with end-over-end mixing at room temperature² for 1 h to overnight³.

3 Collection of Digested Fusion Protein

- Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
- Centrifuge the column at $1000 \times g$ for 1 min to recover the digested fusion protein.
- For maximum recovery of the sample:
 - Seal the spin column with the bottom cap.
 - Add $100 \mu\text{l}$ of digestion buffer.
 - Seal the column and make sure the media is fully resuspended.
 - Remove the bottom cap and place the column in a collection tube. Loosen the top lid.
 - Centrifuge the column at $1000 \times g$ for 1 min to collect the material.
 - Pool the collected fractions.

Notes

1. *Optimization may be required if a digestion buffer other than the recommended buffers is being used.*
2. *Increasing the digestion temperature to 37°C may increase the digestion efficacy for some proteins but may induce artifacts during longer digestion times.*
3. *A shorter incubation time will allow for a more complete coverage of the linker sequence whereas a longer incubation time will reduce the complexity and result in more homogeneous subunits. The incubation time required for complete linker digestion differs between fusion proteins and linkers. The linker may not be completely removed from the linked proteins.*

Quality Control

GlySERIAS Immobilized is tested to meet the specifications and lot-to-lot consistency.

GlySERIAS Immobilized is tested for absence of microbial contamination with blood agar plates, Sabouraud dextrose agar plates and fluid thioglycollate medium.

Related Products

FabRICATOR®

Below hinge digestion of IgG

FabALACTICA®

Above hinge digestion of human IgG1

FabDELLO™

Above hinge digestion of human IgG1, including IgG with mutated hinges

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PRODUCT OVERVIEW



FabRICATOR®
Below hinge
digestion of IgG



FabALACTICA®
Above hinge
digestion of human IgG1



FabDELLO™
Above hinge
digestion of human IgG1



FabRICATOR® Z
Below hinge
digestion of mouse IgG



GingisKHAN®
Above hinge
digestion of human IgG1



FabULOUS™
Above hinge
digestion of IgG



GlyCLICK®
Site-specific
conjugation of IgG



TransGLYCIT™
Transglycosylation
of IgG

ANTIBODY DIGESTION



GlySERIAS™
Hydrolysis of
flexible linkers



GingisREX®
Arginine-specific
protein digestion



GlycINATOR®
Hydrolysis of
all Fc N-glycans



IgGZERO®
Hydrolysis of
Fc N-glycans

ANTIBODY CONJUGATION

FUSION PROTEIN DIGESTION

PROTEOMICS

ANTIBODY DEGLYCOSYLATION



OmniGLYZOR™
Hydrolysis of N- and
mucin-type O-glycans



PNGase F
Hydrolysis of
N-glycans



OpeRATOR®
O-glycan-specific
protein digestion



OglyZOR®
Hydrolysis of
core-1 O-glycans



SialEXO®
Hydrolysis of
sialic acids



FucosEXO™
Hydrolysis of
 α 1-2,3,4 fucose



GalactEXO™
Hydrolysis of β 1-3,4-
linked galactose



GalNAcEXO™
Hydrolysis of
 α -linked GalNAcs

GLYCAN PROFILING



GlycOCATCH®
Enrichment of
O-glycopeptides



Anti-FabRICATOR®
Detection of the
FabRICATOR enzyme



Anti-FabRICATOR® Z
Detection of the
FabRICATOR Z enzyme



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