GlySERIAS™

Immobilized

FOR RESEARCH
USE ONLY

www.genovis.com

STORE AT
+4-8°C

SmartEnzymes™

GENOVIS

INSTRUCTIONS FOR PRODUCTS

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

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

Last revised Feb 2023

Quick Guide

1 Equilibration

- Equilibrate the column with 3 × 300 µl of digestion buffer. Centrifuge at 200 × 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 × g for 1 min to collect the digested fusion protein.
- For maximum recovery, add 100 µl of digestion buffer, resuspend the media and centrifuge at 1000 × 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 $(Gly_4Ser)_n$, 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.

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 µl of digestion buffer[1] 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 × g for 1 min to remove the storage solution.
- Equilibrate the column by adding 300 µl of digestion buffer and centrifuge at 200 × 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 µl 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 temperature[2] for 1 h to overnight[3].

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 × 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 µl 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 × 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

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

ANTIBODY DIGESTION

GingisKHAN®
Above hinge
digestion of human IgG1

FabULOUS™
Above hinge
digestion of IgG

GlyCLICK®
Site-specific
conjugation of IgG

TransGLYCIT™
Transglycosylation
of IgG

ANTIBODY CONJUGATION

GlySERIAS™
Hydrolysis of
flexible linkers

GingisREX®
Arginine-specific
protein digestion

GlycINATOR®
Hydrolysis of
all Fc N-glycans

IgGZERO®
Hydrolysis of
Fc N-glycans

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 α1-2,3,4 fucose

GalactEXO™
Hydrolysis of β1-3,4-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

RESEARCH ANTIBODIES

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