



SUMOstar™ Gene Fusion Technology

NEW METHODS FOR ENHANCING FUNCTIONAL PROTEIN
EXPRESSION AND PURIFICATION IN INSECT CELLS

White Paper

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Introduction

Expressing and purifying large quantities of functional properly folded protein is a bottleneck in structural and functional genomic studies. Expression difficulties typically include poor yield and formation of insoluble aggregates. These issues compel scientists to use gene fusion technologies to increase protein expression. Gene fusion systems such as glutathione-S-transferase (GST), thioredoxin (Trx), and maltose binding protein (MBP), are hampered by the time-consuming and inefficient process to remove the fusion tag. The proteases used to remove these tags (including thrombin, EK, and TEV protease) recognize short primary sequences, and unfortunately often cleave within the recombinant protein. These protein fusion systems are also hampered by poor protein folding capacity, resulting in formation of improperly folded, inactive proteins that aggregate as insoluble inclusion bodies.

The small ubiquitin-related modifier (SUMO) system introduction provides a solution to many problems encountered when using other gene fusion systems. SUMO is a ubiquitin family protein that has several known cellular functions, including enhancing the stability of proteins to which it binds. The SUMO system increases expression levels of many different proteins and provides distinct advantages over other gene fusion systems for several reasons:

1. SUMO increases solubility and stability of recombinant proteins more than other fusion tags.
2. A highly specific, stable, and active SUMO protease is available for efficient fusion tag cleavage and subsequent purification of the recombinant protein.
3. Native N-termini of recombinant proteins are preserved following SUMO protease cleavage.

Furthermore SUMO protease recognizes the tertiary SUMO structure with high specificity, thereby never cleaving within the protein of interest.

The SUMO fusion system sets itself apart from other fusion systems by enhancing the solubility, stability, and functionality of many proteins. Unfortunately, SUMO proteases are expressed in eukaryotic systems, limiting the utility of this fusion tag (until now) to expression and purification of recombinant proteins in prokaryotes. This paper describes the new SUMOstar fusion technology, which allows scientists to reap the benefits of SUMO technology while generating functional proteins in eukaryotic expression systems.

SUMOstar Gene Fusion Technology

SUMO Fusions Protein are Cleaved in Eukaryotic Cells: SUMOstar Technology Rationale

Recombinant protein expression in eukaryotic systems provides the proper protein folding and post-translational modification environment required for functional eukaryotic protein production. Unfortunately, endogenous SUMO protease activities preclude the use of existing SUMO fusion systems in eukaryotes. Figure 1 shows that a SUMO-GFP fusion expressed in Sf9 insect cells is rapidly cleaved by endogenous SUMO protease activity. However, use of a new SUMO family tag—termed SUMOstar—results in greatly enhanced expression of SUMOstar tagged GFP with very little cleavage by endogenous SUMO proteases (Figure 1).

Introduction of SUMOstar Technology: The Concept

LifeSensors has taken an exciting new approach to exploit the solubility, expression, and purification advantages of the SUMO system in eukaryotic hosts. Mutations were introduced into the SUMOstar tag to make it resistant to hydrolysis by endogenous SUMO proteases. Remarkably, the SUMOstar tag has the same solubility, chaperoning, and expression enhancing advantages in eukaryotes as the previous SUMO fusions exhibit in prokaryotes. A SUMOstar protease was also engineered to specifically cleave the SUMOstar tag and generate functional proteins with their native N-termini in all expression hosts.

SUMOstar Expression in Sf9 Insect Cells Using a Baculovirus System:

Insect cells were chosen for protein expression because of their ability to properly fold large amounts of functional eukaryotic proteins. Figure 2 outlines the cloning paradigm for expression of SUMOstar-tagged protein constructs in SF9 insect cells. cDNAs of interest are cloned into the pFastBac vector (Invitrogen) in-frame with the His₆-SUMO tag and expressed under the control of the insect polyhedron promoter. Next, baculovirus are produced, harvested, and used to infect Sf9 insect cells for high level expression of the His₆-SUMO-protein.

SUMOstar Produces Enhancement of Functional Proteins in Both Eukaryotes and Prokaryotes:

A key advantage of the SUMOstar tag is its resistance to cleavage by endogenous eukaryotic SUMO proteases. However, for overall system versatility a comparison between SUMO and SUMOstar tags was made in prokaryotic cells. SUMO-GFP and SUMOstar-GFP were expressed in the *E. coli* Rosetta pLysS strain. After induction, cells were lysed and separated into soluble and insoluble fractions. Equal amounts of protein were resolved on SDS-PAGE and stained with Coomassie blue (Figure 3). These data demonstrate that SUMOstar fusion produces as much or more GFP as the SUMO fusion. These data indicate that the SUMOstar fusion system is also useful for protein expression and solubility enhancement in prokaryotes.

SUMO Tag is Cleaved in Eukaryotes While SUMOstar Tag is Not Cleaved

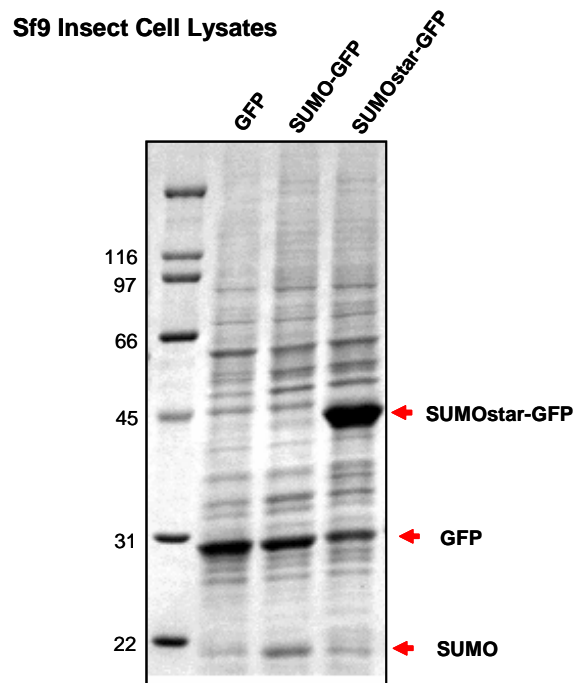


Figure 1.

Baculovirus Mediated Expression of SUMOstar

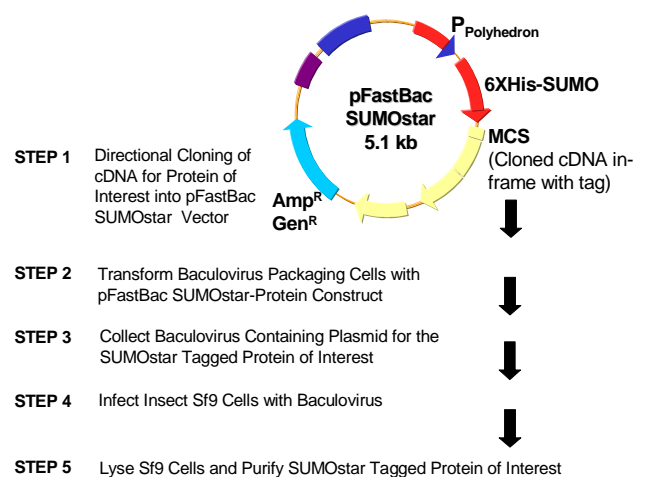


Figure 2.

The SUMOstar Tag is Rapidly and Robustly Cleaved by SUMOstar Protease, Generating Proteins with the Desired N-Terminus:

SUMOstar protease is a very efficient and robust enzyme, which specifically cleaves the SUMOstar tag from the recombinant protein of interest. Equal amounts of Ni-NTA purified His₆-SUMOstar-GFP were digested for 1 hr at 30° C with increasing concentrations of SUMOstar protease and resolved using SDS-PAGE (Figure 4). SUMOstar protease efficiently and completely cleaves the His₆-SUMOstar tag from the protein of interest. Importantly, the SUMO systems (including SUMOstar) have the unique ability to produce purified, functional proteins with the native N-terminal amino acid following protease cleavage.

SUMOstar Enhances Both Intracellular and Secreted Protein Expression in Insect Cells:

Some proteins are normally expressed intracellularly while other proteins are typically secreted into the medium. Versatile systems enhance expression in both contexts. In the first experiment, Sf9 insect cells were infected with baculovirus for expression of His₆-UBP43 (an unstable de-ubiquitinating enzyme), or His₆-SUMOstar-UBP43. Cells were lysed and intracellular proteins were resolved by SDS-PAGE then stained with Coomassie blue (Figure 5A). These data indicate that SUMOstar enhances UPB43 intracellular expression ~5-fold over the His₆ tag alone. Subsequent analysis revealed that UB43 was highly active (data not shown). In a second experiment, trypsin, a common serine proteinase, attached to gp67 (a secretion tag) and gp67-SUMOstar-trypsin were used to test the effect of SUMOstar on secreted proteins. Conditioned medium was collected from Sf9 cells. Secreted proteins were resolved on SDS-PAGE and stained with Coomassie blue (Figure 5B). These data demonstrate that SUMOstar enhances expression of secreted trypsin. Trypsin activity was demonstrated with trypsin purified using the SUMOstar fusion tag (data not shown). Overall, SUMOstar is a powerful system for enhancing yield and solubility of intracellular and secreted functional proteins in insect cells.

SUMOstar Technology Provides an Easy Method for Purifying Untagged Eukaryotic Proteins:

A His₆ tag is combined with the SUMOstar fusion tag to facilitate protein purification using immobilized metal affinity chromatography (IMAC). Figure 6 shows SDS-PAGE analysis of fractions from the purification of SUMOstar-UBP43 from an insect cell lysate. SUMOstar-UBP43 yield, recovery, and purity are all high after elution.

Four Steps to Pure, Untagged Functional Protein: Protein purification and fusion tag removal is an easy and efficient process using the SUMOstar system. The process involves an initial purification of His₆-SUMOstar-Protein from the crude cell lysate using an IMAC column. The tagged protein can then either be: 1) directly used in an assay or 2) cleaved with SUMOstar protease to remove the fusion tag. Both SUMOstar protease and the cleaved tag contain a His₆ moiety; therefore, purified untagged protein is obtained by running the protease reaction on a second IMAC column (to bind the tag and protease) and collecting the flow

SUMOstar Expression in Prokaryotes

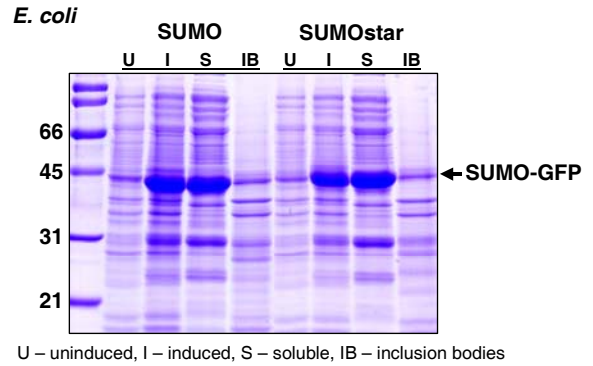


Figure 3.

SUMOstar Protease Efficient Cleavage

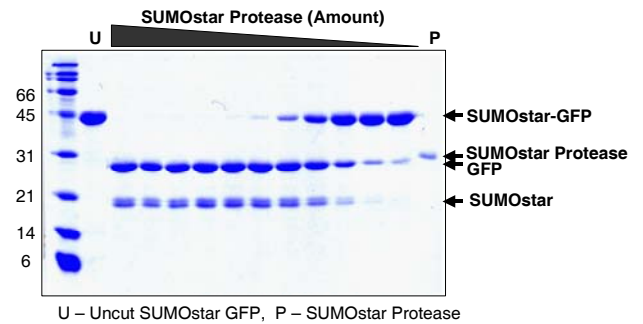


Figure 4.

SUMOstar Enhances Intracellular and Secreted Protein Expression

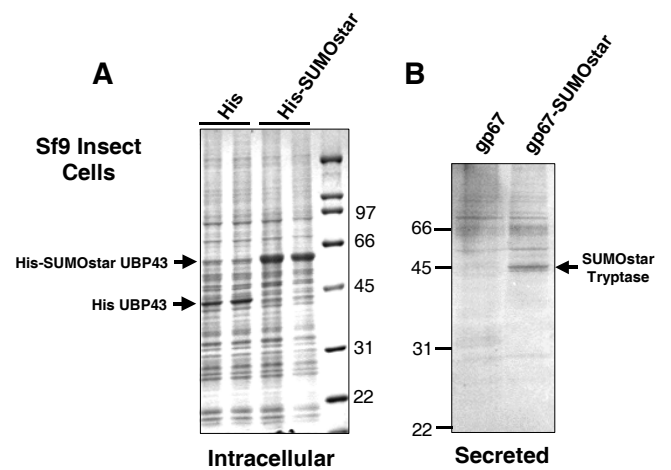


Figure 5.

through (protein).

SUMOstar: A Versatile Protein Expression and Purification Tool:

The SUMOstar system is your answer to protein expression and purification questions. SUMOstar, unlike other fusion tags, produces higher yield, and more stable, soluble, and functional proteins. Unique to the SUMO systems are: 1) generation of native N-terminal amino acid (except proline) after fusion tag cleavage and 2) a specific protease that recognizes the tertiary structure of SUMO and never cleaves within the protein of interest. While SUMO fusion proteins produce outstanding enhancement of proteins in prokaryotic hosts, SUMOstar brings the SUMO expression advantages to eukaryotes. SUMOstar makes protein expression and purification better and simpler and allows you to focus on other questions.

The SUMOstar and SUMO fusion technology platforms continue to develop, as scientists search for improved processes to express and purify functional proteins. Our methodologies and insight also expands and evolves on a daily basis. Please check our website (www.lifesensors.com) for recent developments and frequent updates.

Frequently Asked Questions

Q: Does LifeSensors have patent(s) on this technology?

A: LifeSensors has worldwide patents and patent applications on the use of SUMOstar-fusion and applications of the SUMOstar tag and SUMOstar proteases

Q: Is LifeSensors marketing a vector, a specific protease, or kits containing all necessary components?

A: LifeSensors has developed a complete SUMOstar kit for eukaryotic and prokaryotic systems. LifeSensors, Inc. offers a broad range of services and support for various applications of the SUMOstar technology.

Q: How specific is the enzyme?

A: SUMOstar protease is a highly specific enzyme – it recognizes the tertiary SUMOstar structure. SUMOstar protease consistently cleaves at the junction between SUMOstar tag and the protein of interest to deliver a protein with its native N-terminus.

Q: Will commercially available SUMO Protease 1 cleave the SUMOstar tag?

A: No, the SUMOstar tag is only cleaved by SUMOstar Protease.

Q: Is SUMOstar protease as robust as commercially available SUMO protease 1?

Purification of SUMOstar Constructs

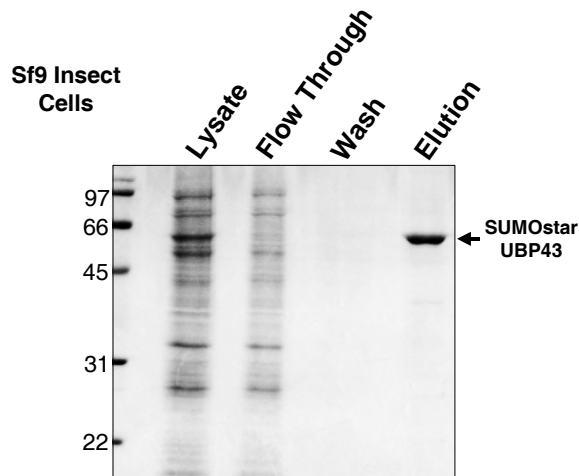


Figure 6.

A: SUMOstar protease is highly active and the cleavage rate is very similar to SUMO protease 1. Cleavage by SUMO protease (like SUMO protease 1) generates the native N-terminal amino acid (except for proline).

Q: What is the size of SUMOstar?

A: SUMOstar is 100aa or 11.5 kDa. However, it migrates aberrantly at approximately 20 kDa on an SDS-polyacrylamide gel.

Q: Will SUMO antibody detect SUMOstar fusions?

A: SUMO antibody can potentially detect SUMOstar fusions; however for the greatest detection we recommend using SUMOstar antibody that has been specifically engineered to provide the greatest detection for SUMOstar fusions.

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