

SUMO Fusion Platform

-----enhanced functional protein expression in bacterial cells.

Features:

- **Increased solubility and stability**
- **Enhanced protein folding**
- **Production of extremely toxic proteins**
- **Simple and efficient purification**
- **Complete pinpointed tag removal**

Creative Biolabs has established a unique SUMO Fusion Platform that allows production of large quantities of biologically active proteins in bacterial cells with their native N-terminus intact. SUMO (small ubiquitin-like modifier), as a fusion tag, can significantly enhance functional protein expression by improving protein folding, solubility and stability in prokaryotes and simplifying protein purification. Importantly, SUMO technology enables pinpointed and complete removal of the SUMO tag from any recombinant proteins. In addition a *lac* operon system was integrated into the platform and glucose can be employed to completely shut off the basal/leaky expression of target proteins before IPTG induction. As a result, SUMO Fusion Platform also permits efficient expression of extremely toxic proteins. For the same reason, target protein expression on this platform can be automatically induced without using IPTG if both glucose and lactose are included in the culture medium.

The principle behind SUMO Fusion Technology

In cells, proteins are tagged for degradation by ubiquitin and targeted to the 26S proteasome. In contrast, covalent modification of cellular proteins by the ubiquitin-like modifier SUMO regulates various cellular processes, such as nuclear transport, signal transduction, and protein stabilization. Ubiquitin-like proteins fall into two classes: the first class, ubiquitin-like modifiers (UBLs) function as modifiers in a manner analogous to that of ubiquitin. Examples of UBLs are SUMO, Rub1 (also called Nedd8), Apg8, and Apg12. The second class of proteins includes parkin, RAD23, and DSK2 and are designated ubiquitin-domain proteins (UDPs). These proteins contain domains that are related to ubiquitin but are otherwise unrelated to each other. In contrast to UBLs, UDPs are not conjugated to other proteins. Once covalently attached to cellular targets, SUMO regulates protein-protein and protein-DNA interactions, as well as localization and stability of the target protein. Sumoylation occurs in most eukaryotic systems, and SUMO is conserved from yeast to humans. SUMO and ubiquitin only show about 18% homology, but both possess a common three-dimensional structure characterized by a tightly packed globular fold with β -sheets wrapped around an α -helix.

It has been known for a long time that ubiquitin exerts chaperoning effects on fused proteins in *E. coli* and yeast, increasing their yield and solubility. Attachment of a highly stable protein (ubiquitin or SUMO) at the N-terminus of a partner protein increases the yield of the recombinant fusion protein. The enhanced solubility demonstrated by fusing ubiquitin and ubiquitin-like moieties to the N-terminus of the protein-of-interest may be explained by the outer hydrophilicity and inner hydrophobicity of the folded structure of ubiquitin and SUMO, exerting a detergent-like effect on less soluble fusion partner proteins.

The SUMO Fusion Platform

While ubiquitin fusion has been known for many years to enhance protein expression, its utility as a protein purification modality is compromised by the inefficient nature of ubiquitin hydrolase, a protease – the enzyme that releases the partner protein from ubiquitin by hydrolysing the peptide bond. Likewise, commonly

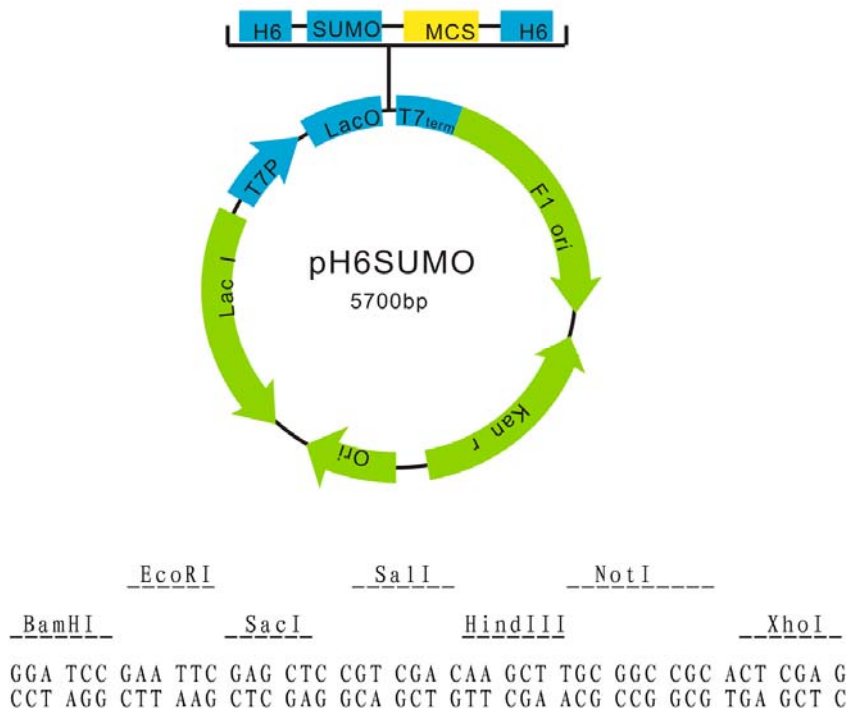
used proteases such as thrombin, enterokinase, rhinovirus proteases, and TEV, do not cleave all fusion proteins efficiently and, moreover, can generate unnatural N-termini by leaving some amino acids from the cleavage recognition site. In eukaryotic cells ubiquitin is not a convenient tag since ubiquitinated proteins are targets for the degradation machinery.

Recently, Ulp1 core (SUMO Protease 1), a yeast SUMO protease equivalent of an ubiquitin protease, has been evaluated as a tool for purification of recombinant SUMO fusion proteins expressed in *E. coli*. SUMO protease 1 has unique advantages when compared with other proteases commonly used in recombinant protein production. SUMO Protease 1 recognizes the Smt3 structure at the N-terminus of the partner protein and cleaves the junction irrespective of the N-terminal sequence of the protein (except proline). Also, SUMO Protease 1 never cleaves within the protein-of-interest. This robust SUMO protease can efficiently and specifically cleaves the target protein off the fusion tag without leaving or moving any extra amino acids.

With the advent of this valuable platform, we are now able to maximize the production of functional, properly-folded recombinant proteins with the fusion tag completely and accurately removed.

Experimental Protocol

- 1) Insertion of genes encoding protein of interest into the in-house SUMO fusion expression vector, pH6SUMO.



- 2) Scale-up expression with fermentors (50L, 20L or 3.7 L)
- 3) Extraction of proteins of interest by French Press
- 4) Industrial purification of proteins of interest by IMAC (Immobilized Metal Affinity Chromatography)
- 5) Tag removal with robust in-house SUMO protease
- 6) Isolation of tag free proteins of interest by one step of IMAC

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