Champion™ pET Expression with SUMO™ tag enables high-level soluble expression and purification of recombinant proteins and peptides with native N-termini in E. coli.

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Abstract

The Champion™ pET SUMO Expression System produces high levels of soluble protein in bacteria. It utilizes a small ubiquitin-related modifier (SUMO) fusion, belonging to the growing family of ubiquitin-related proteins, to enhance the solubility of expressed fusion proteins. In contrast to ubiquitin, SUMO is involved in the stabilization and localization of proteins *in vivo*. After expression, the 13-kd SUMO moiety can be cleaved by the highly specific and active SUMO (ULP-1) protease at the carboxyl terminal, producing a native protein*. The BL21(DE3) expression strain, included in the kit, maximizes expression of soluble protein. The Champion™ pET SUMO Peptide and Protein Expression System features:

•Enhanced solubility with an N-terminal SUMO fusion

•Highly efficient cleavage of native protein of interest with SUMO (ULP-1) protease*

•T7*lac* promoter for high-level protein expression

•N-terminal 6xHis tag for protein detection and purification

•BL21(DE3) expression strain for maximizing expression of soluble protein

Introduction

T7-based *E.Coli* expression systems are widely employed to produce high yields of protein with a relatively low investment of funding and work hours. However, most plasmid-based expression systems utilize fusion tags and cloning methodologies that result in additional residues in the final purified protein. The pET champion SUMO protein expression system is specifically designed to provide high-level expression of proteins and peptides with native n-termini. This is accomplished by the remarkable specificity and efficiency of the SUMO protease enzyme (ULP1). Unlike other proteases used for fusion tag removal, SUMO protease recognizes the entire face of the SUMO moiety and cleaves it reliably and specifically from the protein of interest in a wide range of temperature and buffer conditions. The seamless cleavage of the fusion protein and the efficiency and specificity of the SUMO protease are great strengths of the system which are currently unmatched by other fusion-tag based *E. coli* expression schemes.

To utilize the SUMO expression system, the customer clones their gene of interest onto the pET SUMO expression vector via T/A ligase technology and proceeds to express the protein of interest with the SUMO (SMT3) moiety as an n-terminal fusion partner. SUMO, a highly soluble and monomeric protein, can lead to enhanced solubility of the recombinant proteins that it is fused to. The SUMO tag can then be liberated from the protein of interest by cleavage with the SUMO protease in a simple enzymatic reaction at 4°C-30°C. The customer will obtain their protein of interest with the desired native n-terminus free from any cleavage sites or extra residues resulting from DNA cloning strategies*.

*Fusion partners with an n-terminal proline residue require additional serine spacer between SUMO and the fusion partner for cleavage.

Figure 1. Map of Champion pET SUMO T/A Vector. Features of the pET SUMO vector include T7*lac* promoter for high-level protein expression and n-terminal HIS tag for purification and detection of fusion proteins. SUMO and HIS tags can be successfully cleaved from most substrates to yield a native n-terminus

Results

Expression profiles of test genes with and without the n-terminal SUMO fusion partner were examined.

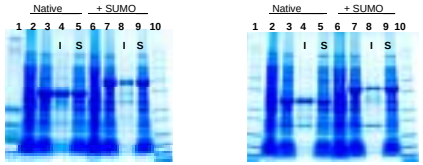

Figure 2. Expression comparison of two test proteins with and without the SUMO tag in BL21 (DE3) Two test proteins were expressed in native state and with SUMO tag. Cells were lysed and fractionated, then visualized on 4-20% Tris Glycine SDS-PAGE gel. Lanes 2 and 6 show un-induced whole cell lysate, Lanes 3 and 7 show induced whole cell lysate, "I" denotes the insoluble fraction, "S" denotes the soluble fraction.

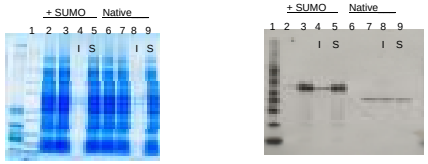

Figure 3. Expression comparison of test protein with and without the SUMO tag in BL21 (DE3) pLysS Test protein was expressed with and without SUMO-tag. Cells were lysed and fractionated, then visualized on 4-20% Tris-Glycine SDS-PAGE gel. 25X diluted samples were ran and analyzed via Western blot with 6HIS antibody. Lanes 2 and 6 show un-induced whole cell lysate, Lanes 3 and 7 show induced whole cell lysate, "I" denotes the insoluble fraction, "S" denotes the soluble fraction.

Effect of n-terminal residue of the cloned fusion partner on SUMO protease activity was investigated with a series of n-terminal mutant CAT fusion proteins.

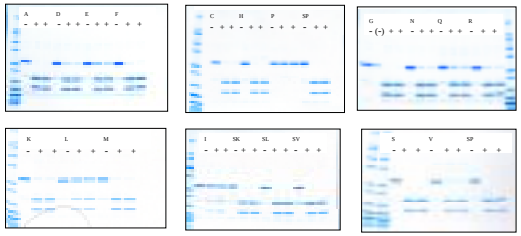

Figure 4. Effect of n-terminal (P'1) residue of fusion partner on SUMO protease cleavage . A series of mutants of the CAT (Chloramphenicol Acetyltransferase) was created by altering the n-terminal codon of the PCR product cloned into the SUMO vector. All fusions were purified using IMAC batch format. All 20 amino acid residues were tested for cleavage with SUMO protease for 1 and 2 hours at 30°C. Letters designate n-terminal residue of CAT fusion partner. Only proline resisted cleavage. Addition of a serine spacer facilitates cleavage of difficult or un-cleavable fusion junctions by the SUMO protease.

Ni-NTA resin can be used to separate purified fusion partner from 6X HIS-tagged components of the protease reaction in a simple benchtop format.

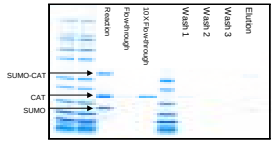

Figure 5. Nickel resin clean-up of SUMO Protease reaction in batch (gravity flow) format. SUMO-CAT expression control was incubated with SUMO protease overnight at 4°C. The Protease reaction was then diluted 8X and bound to Ni-NTA resin. The purified CAT protein was collected in the flow-through fraction and concentrated to 10X volume by centrifugal concentration (Amicon Ultra 10K MWCO) The 6XHIS-tagged SUMO Moiety, un-cleaved fusion, and SUMO Protease are bound to Ni-NTA resin.

SUMO Protease cleavage of purified SUMO—peptide fusions.

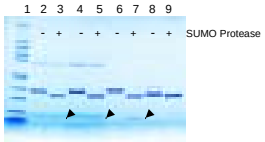

Figure 5: Sumo Protease cleavage of SUMO-peptide fusions.
IMAC purified SUMO-peptide fusions were incubated with SUMO protease for 3 hours at 16°C, then visualaized on 4-12% NuPAGE SDS-PAGE gel. Lanes 2-7 show three independent SUMO-sarcotoxin clones +/- SUMO protease. Lanes 8-9 show SUMO-V5 fusion +/- SUMO Protease. Arrowheads indicate position of cleaved sarcotoxin peptide (approx. 4 kDa). The smaller molecular weight of the V5 epitope (approx. 1 kDa) precluded analysis by gel.

RP-HPLC purification and MALDI-TOF analysis of peptides following cleavage of SUMO fusions.

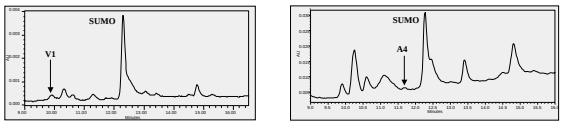

Figure 6. RP-HPLC purification of cleaved peptides.
Reverse phase HPLC traces showing separation of V5 and sarcotoxin peptides from SUMO moiety following cleavage by SUMO Protease.

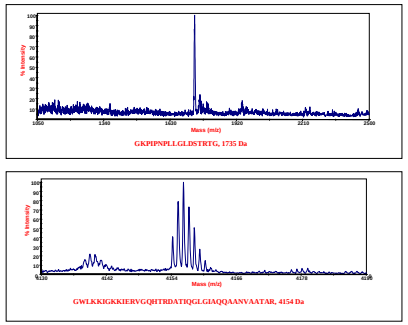

Figure 7. Mass Spectrometry traces of purified peptides.
HPLC fractions containing purified peptide were analyzed by DE-STR MALDI-TOF instrument using a-cyano-4-hydroxycinnamic acid (CHCA) as matrix. Intact V5 (1734 Da) and Sarcotoxin (4154 Da) peptides were successfully purified.

Conclusion

The Champion pET SUMO Protein Expression System offers a new and important tool for high-level soluble expression of proteins and peptides with native n-termini. The companion SUMO Protease enzyme works with the great specificity and efficiency on most substrates in a wide range of temperatures without undue damage to the protein of interest. Customers who are already familiar with the use of T/A vectors as well as T7-based expression in BL21 (DE3) should have few worries about working this powerful new expression system into their research.

References

1. Mossessova, E. and Lima, C.D. Molecular Cell, 5:865-876, 2000.
2. Skosyrev, V.S. et. al, Expression of the recombinant antibacterial peptide sarcotoxin IA in *Escherichia coli* cells, Protein Expression & Purification , 28:350-356, 2003.
3. Studier, F.W., Rosenberg, A. H., Dunn, J. J. and Dubendorff, J. W. (1990) *Meth. Enzymol.* 185:60-89.

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