

PROTEIN TAGS AND LEADER SEQUENCES

Introduction

In this document there is summary information about

- Commonly used protein tags
- Secretory leader sequences used for expression in either insect or mammalian cells

Protein tags

AVI tag	Applications	Biotinylation on lysine
Comments	In the presence of biotin and ATP, biotin ligase catalyses amide linkage between the biotin and the specific lysine of the 15-aa AviTag peptide.	
Sequence	GLNDIFEAQKIEWHE	

FLAG tag	Applications	Affinity purification, detection
Comments	Detected using anti FLAG antibody Affinity purified using anti-FLAG linked to agarose resin, eluted with FLAG peptide.	
Sequence	DYKDDDDK	

Haemagglutinin (HA) tag	Applications	Detection
Comments	Detected using anti-HA antibody. Amino acids 98 – 106 from human influenza haemagglutinin	
Sequence	YPYDVPDYA	

His tags	Applications	Affinity purification, detection
Comments	Detected using anti-His antibody Affinity purified using Metal chelate (usually nickel) agarose resin, eluted with imidazole The histidine affinity tag (HAT) tag is derived from chicken lactate dehydrogenase.	
Sequences		
6His version (most common)	HHHHHH	
6x HisAsn	HNHNHNHNHNHN	
Histidine affinity tag (HAT)	SLKDHLIHNVHKEEHAHAHNK	

Myc tag	Applications	Detection
Comments	Detected using anti-Myc antibody (9E10) Derived from c-myc gene product	
Sequence	EQKLISEEDL	

Strep tag II	Applications	Affinity purification
Comments	WSHPQFEK	
Sequence	Affinity purified using Strep-Tactin or Strep-Tactin XT resin. Eluted with desthiobiotin	

Twin strep tag II	Applications	Affinity purification
Comments	Affinity purified using Strep-Tactin or Strep-Tactin XT resin. Eluted with desthiobiotin	
Sequence	WSHPQFEKGSAGSAAGSGAGWSHPQFEK	



Glutathione S Transferase (GST)	Applications	Affinity purification, detection, improved solubility
Comments	Affinity purified using glutathione sepharose resin. Eluted with glutathione. UniProt ID: P08515	
Sequence		
MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQS MAIRYIADKHNMLGGCPKERAIEISMLEGAVLDIRYGVSRAYS KDFETLKVDFLSKLPEMLKMFEDRL CHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSSKYIAWPLQG WQATFGGGDHPPK		

Maltose binding protein (MBP)	Applications	Affinity purification, improved solubility	detection,
Comments	Affinity purified using amylose resin. Eluted with maltose. UniProt ID:P0AEX9		
Sequence			
KIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRFGG YAQSGLLAEITPDKAFQDKLYPFTWDVAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKEL KAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVNDAGAKAGLTFLVDLIKNNKHMNA DTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNK ELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYYEELAKDPRIAATMENAQKGEIMPNI PQMSAFWY AVRTAVINAASGRQTVDEALKDAQTRITK			

Mutant Maltose binding protein (MBP)	Applications	Affinity purification, Improves solubility	Detection,
Comments	Affinity purified using amylose resin. Eluted with maltose. Based on UniProt ID: P0AEX9 367aa		
Sequence			
KTEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRFG GYAQSGLLAEITPAAAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKEL LKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYAAGKYDIKDVGVNDAGAKAGLTFLVDLIKNNKHMN ADTDYSIAEHAFNHGETAMTINGPWAWSNIDTSAVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPN KELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYYEELVKDPRVAATMENAQKGEIMPNI PQMSAF WYAVRTAVINAASGRQTVDAALAAQTN			



Small Ubiquitin-like Modifier (SUMO or SUMO Pro alternative)	Applications	Improve expression levels and solubility
Comments	Gives clean N- terminus post cleavage with Ulp1 protease Based on UniProt ID Q12306 (from <i>Saccharomyces cerevisiae</i>) SUMO Pro has [T77A] mutation and GSLQ- at N- terminus. Used in Invitrogen pET SUMO Check fusion doesn't start with Pro, Lys, Val or Leu - if so add in Ser to start	
Sequences (1. Original SUMO tag, 2. SUMO Pro alternative)		
1. MSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGGKEMDSLRFlyDGIRIQADQTPEDLDMEDNDIIEAHREQIGG 2. GSLQDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGGKEMDSLRFlyDGIRIQADQAPEDLDMEDNDIIEAHREQIGG		

Small Ubiquitin-like Modifier (SUMO) Star	Applications	Improve expression levels and solubility
Comments	Gives clean N- terminus post cleavage with Ulp1 protease Licensed by Lifesensors Inc For use in insect, yeast and mammalian cells that contain endogenous de-SUMOylases. R64T, R71E double mutant is resistant. Lifesensors sell a mutated Ulp1 SUMOStar protease that recognises and cleaves SUMO Star. Check fusion doesn't start with Pro, Lys, Val or Leu - if so add in Ser to start	
Sequence		
GSLQDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQGGKEMDSLTFLYDGIEIQADQAPEDLDMEDNDIIEAHREQIGG		



Fc tag	Applications	Affinity purification, detection
Comments	Affinity purified using protein A (or similar) resin. Eluted with acid pH. Fc from Human IgG1 (Uniprot: P01857) is the most commonly used Fc tag. However others can be used including:- Human: IgG2-Fc, IgG3-Fc, IgG4-Fc Mouse: IgG1-Fc, IgG2a-Fc, IgG2b-Fc, IgG3-Fc Rat: IgG1-Fc, IgG2a-Fc, IgG2b-Fc, IgG2c-Fc Rabbit: IgG-Fc Canine: IgG-Fc	
Sequence		
EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK		



Secretory signal sequences

Honey bee melittin leader	Applications	Secreted expression
Comments	Target protein is secret into the media when expressed in insect cell culture	
Sequence	MKFLVNVALVFMVVYISYIYA	

Ig kappa leader	Applications	Secreted expression
Comments	Target protein is secret into the media when expressed in mammalian culture	
Sequence	METDTLLLWVLLLWVPGSTG	

Azurocidin leader	Applications	Secreted expression
Comments	Target protein is secret into the media when expressed in mammalian culture. Especially beneficial for CHO cell expression	
Sequence	MTRLTVLALLAGLLASSRA	

PeIB leader sequence	Applications	Periplasmic expressionn
Comments	Target protein is secret into the periplasm when expressed in bacterial culture	
Sequence	MKYLLPTAAAGLLLLAAQPAMA (UniProt Q04085).	

DsbA leader sequence	Applications	Periplasmic expressionn
Comments	Target protein is secret into the periplasm when expressed in bacterial culture	
Sequence	MKYLLPTAAAGLLLLAAQPAMA	

OmpA leader sequence	Applications	Periplasmic expressionn
Comments	Target protein is secret into the periplasm when expressed in bacterial culture	
Sequence	MKKTAIAIAVALAGFATVAQA	



References

Detailed review on protein tags [1]

[1] M. E. Kimple, A. L. Brill, and R. L. Pasker, 'Overview of Affinity Tags for Protein Purification', *Curr. Protoc. Protein Sci.*, vol. 73, no. 1, Aug. 2013, doi: 10.1002/0471140864.ps0909s73.

[2] Sockolosky, J. T; Szoka, F. C (2012). "[Periplasmic production via the pET expression system of soluble, bioactive human growth hormone](#)". *Protein Expression and Purification*. **87** (2): 129–135. doi:10.1016/j.pep.2012.11.002. [PMC 3537859](#). [PMID 23168094](#).

[3] SP Lei et al., [Characterization of the Erwinia carotovora pelB gene and its product pectate lyase](#). *J. Bacteriol.* 169(9): 4379–4383 (1987).

