

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	

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)	SLKDHLIHNVHKEEHAHANCK	

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

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	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKTQSMARIYIAD KHNMLGGCPKERAEISMLEGAVLDIRYGVSR IAYSKDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDHVTHP DFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSSKYIAWPLQG WQATFGGGDHPPK	

Maltose binding protein (MBP)	Applications	Affinity purification, detection, improved solubility
Comments	Affinity purified using amylose resin. Eluted with maltose. UniProt ID:P0AEX9	
Sequence	KIEEGKLIWINGDKGYNGLAIEVGGKFEKDTGIKVTVEHPDKLEEFQVAATGDGPDIIFWAHDRFGGYAQSG LLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALS LIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNL QEPYFTWPLIAADGGYAFKYENGYDIKDVGVNDAGAKAGLFLVDLIK NKHMNADTDYSIAEAAFNKGETAM TINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPL GAVALKSYEEELAKDPRIAATMENAQKGEIMPNI PQMSAFWYAVRTAVINAASGRQTVDEALKDAQTRITK	

Mutant Maltose binding protein (MBP)	Applications	Affinity purification, Detection, Improves solubility
Comments	Affinity purified using amylose resin. Eluted with maltose. Based on UniProt ID: P0AEX9 367aa	
Sequence		
KTEEGKLVIIWINGDKGYNGLAEVGGKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRFGGYAQSG LLAEITPAAAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNL QEPYFTWPLIAADGGYAFKYAAGKYDIKDVGVNDNAGAKAGLTFVLDLIKHKHMNADTDYSIAEHAFNHGETA MTINGPWAWSNIDTSAVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDK PLGAVALKSYEEELVKDPRVAATMENAQKGEIMPNIQMSAFWYAVRTAVINAASGRQTVDAALAAAQTN		

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. MSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDSLRFlyDGIRIQ ADQTPEDLDMEDNDIIEAHREQIGG 2. GSLQDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDSLRFlyDGIRI QADQAPEDLDMEDNDIIEAHREQIGG		

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		
GSLQDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDSLTFlyDGIEIQA DQAPEDLDMEDNDIIEAHREQIGG		

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

SECRETORY SIGNAL SEQUENCES

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

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

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.