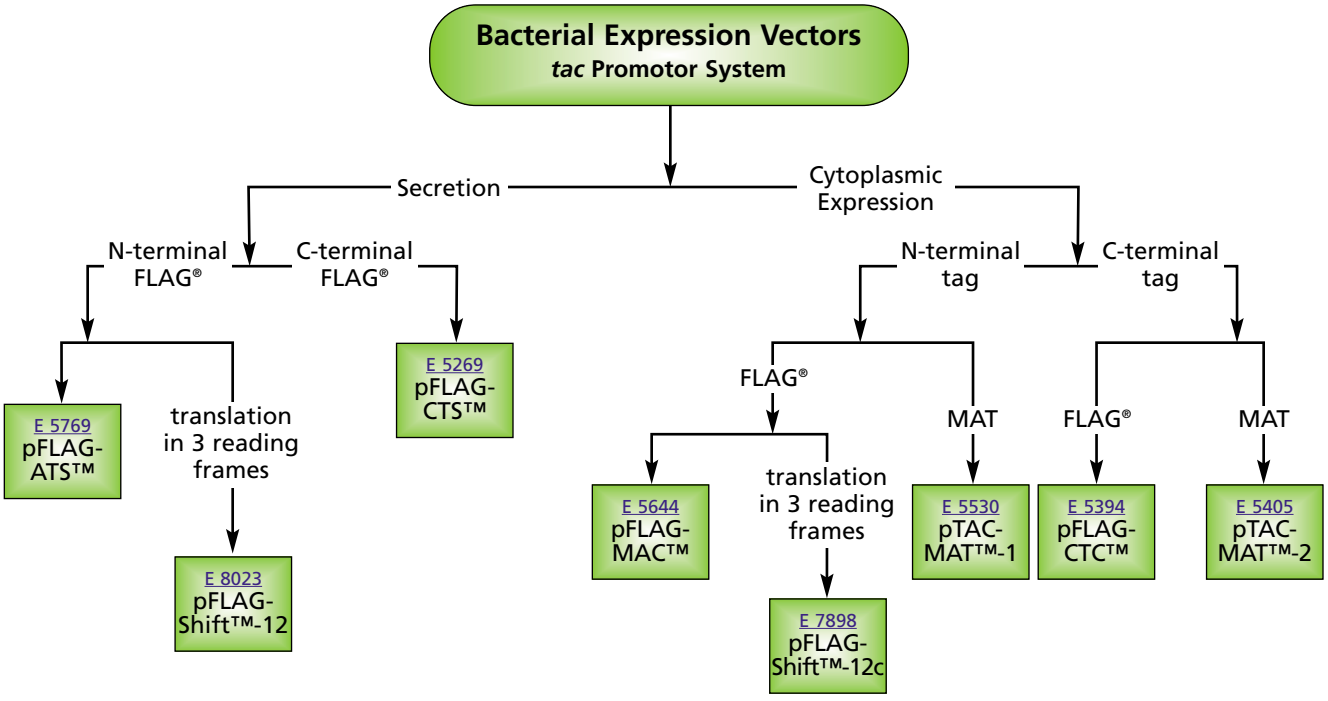
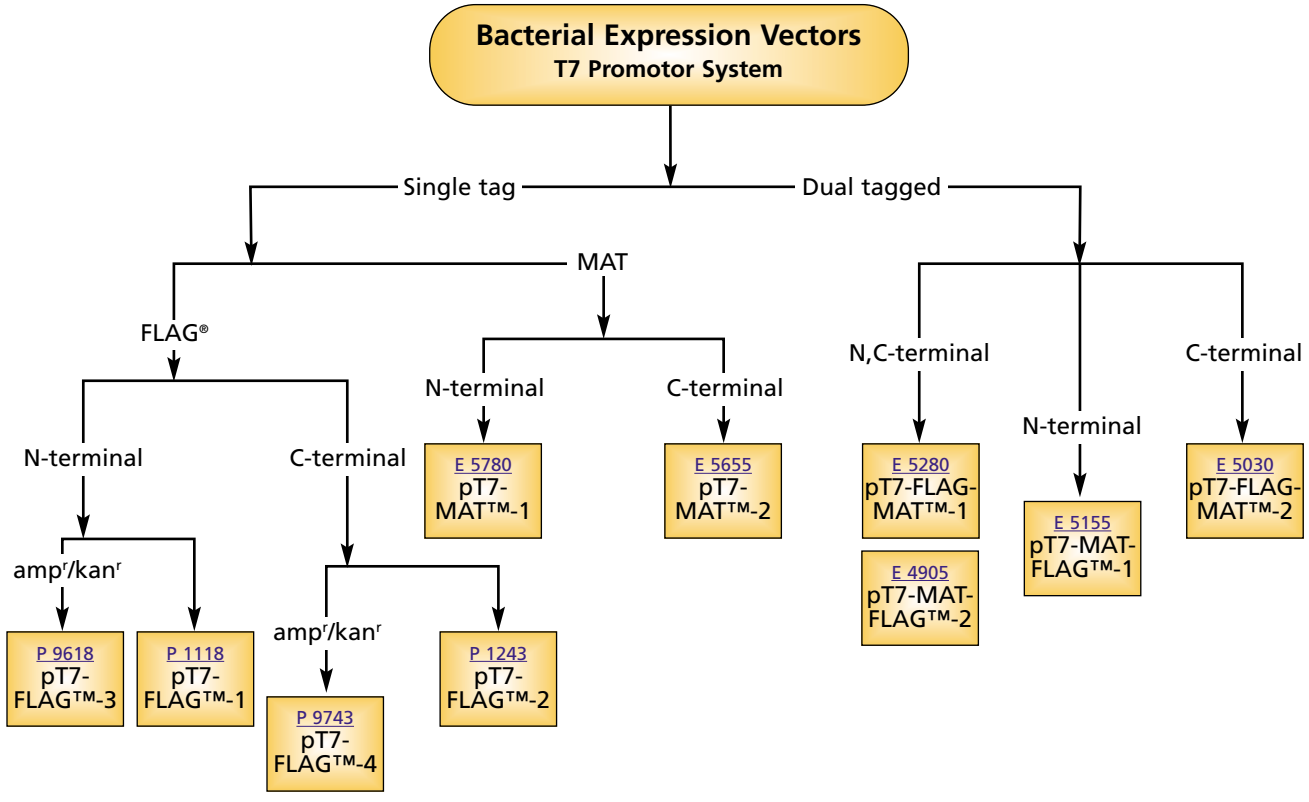
CLONING AND EXPRESSION

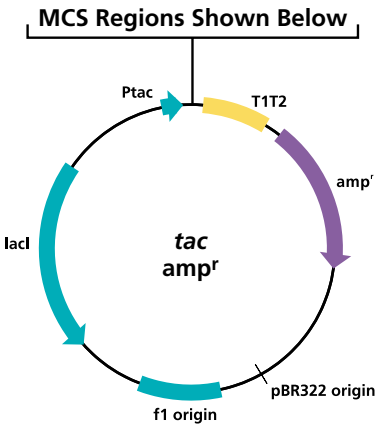
Bacterial Expression Vectors

Bacterial *tac* and T7 promoter-based vectors allow expression, detection and purification of recombinant FLAG® and MAT™ (Metal Affinity Tag) fusions in *E. coli*. Bacterial FLAG® vectors offer a choice of periplasmic (+OmpA) or cytoplasmic expression with either amino- or carboxy-terminal tagging. Several vectors containing the T7 promoter offer dual tag options for FLAG and MAT-tagged fusion proteins. These vectors confer ampicillin resistance for easy selection of positive transformants. Additionally, the vectors contain the *lacI* gene for repression of the *tac* and T7 promoters, the T1T2 transcriptional terminator, the pMB1 (derivative of pBR322) origin of replication, and the f1 origin.





CLONING AND EXPRESSION



Bacterial Expression Vectors

tac Promoter System

Vectors utilizing the strong *tac* promoter (a hybrid of the *E. coli trp* and *lac* promoters) offer protein expression levels in excess of 10 mg/L of culture when using IPTG as a de-repressor. These vectors can be used to express protein in any established *E. coli* expression host.

FLAG-SHIFT™ vectors are available for sequences where the amino-terminus has not been precisely defined. These vectors contain a "shift" sequence that allows expression of an insert in all three reading frames, thus without regard to the reading frame in which it was originally cloned.

pFLAG-ATS™

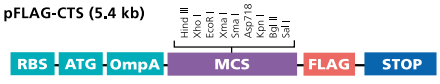
Expression and secretion of N-terminal FLAG fusion proteins under the *tac* promoter. Supplied with a pFLAG-ATS-BAP Control Vector.

Product Code	Description	Size
E 5769	pFLAG-ATS Expression Vector	10 µg

pFLAG-MAC™

Cytoplasmic expression of N-terminal Met-FLAG fusion proteins under the *tac* promoter. Supplied with a pFLAG-ATS-BAP Control Vector.

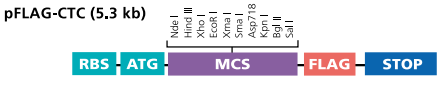
Product Code	Description	Size
E 5644	pFLAG-MAC Expression Vector	10 µg



pFLAG-CTS™

Expression and secretion of C-terminal FLAG fusion proteins under the *tac* promoter.

Product Code	Description	Size
E 5269	pFLAG-CTS Expression Vector	10 µg



pFLAG-CTC™

Cytoplasmic expression of C-terminal FLAG fusion proteins under the *tac* promoter.

Product Code	Description	Size
E 5394	pFLAG-CTC Expression Vector	10 µg

pFLAG-Shift$_{12}$™

Periplasmic expression of N-terminal FLAG fusion proteins under the *tac* promoter. Ideal when the reading frame of the insert is unknown. The FLAG-Shift$_{12}$ DNA sequence causes expression of FLAG fusion proteins in all 3 reading frames. Supplied with a pFLAG-SHIFT$_{12}$-BAP Control Vector.

Product Code	Description	Size
E 8023	pFLAG-Shift$_{12}$ Expression Vector	10 µg

pFLAG-Shift$_{12c}$™

Cytoplasmic expression of N-terminal FLAG fusion proteins under the *tac* promoter. Ideal when the reading frame of the insert is unknown. The FLAG-Shift$_{12}$ DNA sequence causes expression of FLAG fusion proteins in all 3 reading frames. Supplied with a pFLAG-SHIFT$_{12}$-BAP Control Vector.

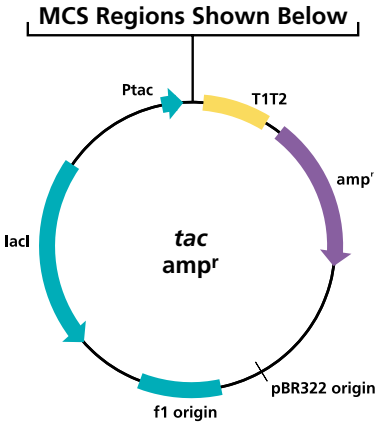
Product Code	Description	Size
E 7898	pFLAG-Shift$_{12C}$ Expression Vector	10 µg

CLONING AND EXPRESSION

Bacterial Expression Vectors

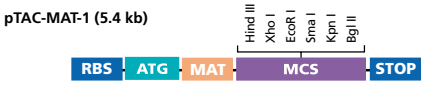
tac Promoter System Continued

The MAT™ tag or Metal Affinity Tag (HNHRHKH) has been created for purification of recombinant MAT fusion proteins using HIS-Select™ Nickel and Cobalt Affinity Gels. HIS-Select products allow for highly selective purification of histidine-tagged fusion proteins such as MAT fusions. Many of our newest vectors make use of the MAT tag, often in combination with the well-known FLAG® tag. MAT tag containing vectors are offered in formats for N-terminal or C-terminal tagging.



pTAC-MAT™-1

Cytoplasmic expression of N-terminal MAT fusion proteins under the *tac* promoter. Supplied with a pTAC-MAT-2+BAP Control Vector.



Product Code	Description	Size
E 5530	pTAC-MAT-1 Expression Vector	10 µg

pTAC-MAT™-2

Cytoplasmic expression of C-terminal MAT fusion proteins under the *tac* promoter. Supplied with a pTAC-MAT-2+BAP Control Vector.

Product Code	Description	Size
E 5405	pTAC-MAT-2 Expression Vector	10 µg

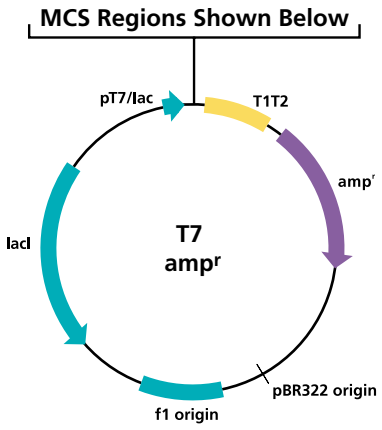
Bacterial Expression Vector Selection Table

***tac* and T7 promoter systems**

Product	Product Name	Promoter	FLAG	MAT	OmpA	Ek Site	amp^r	kan^r
E 5769	pFLAG-ATS	*tac*	N		√	√	√	
E 5644	pFLAG-MAC	*tac*	N			√	√	
E 5269	pFLAG-CTS	*tac*	C		√		√	
E 5394	pFLAG-CTC	*tac*	C				√	
E 8023	pFLAG-Shift$_{12}$	*tac*	N		√	√	√	
E 7898	pFLAG-Shift$_{12C}$	*tac*	N			√	√	
E 5530	pTAC-MAT-1	*tac*		N			√	
E 5405	pTAC-MAT-2	*tac*		C			√	
P 1118	pT7-FLAG-1	T7/*lacO*	N			√	√	
P 1243	pT7-FLAG-2	T7/*lacO*	C				√	
E 5780	pT7-MAT-1	T7/*lacO*		N			√	
E 5655	pT7-MAT-2	T7/*lacO*		C			√	
E 5280	pT7-FLAG-MAT-1	T7/*lacO*	N	C		√	√	
E 4905	pT7-MAT-FLAG-2	T7/*lacO*	C	N			√	
E 5155	pT7-MAT-FLAG-1	T7/*lacO*	N	N		√	√	
E 5030	pT7-FLAG-MAT-2	T7/*lacO*	C	C			√	
P 9618	pT7-FLAG-3	T7/*lacO*	N			√	√	√
P 9743	pT7-FLAG-4	T7/*lacO*	C				√	√

N = N-terminal tag C = C-terminal tag OmpA = periplasmic localization Ek = Enterokinase cleavage site
amp^r = ampicillin resistance gene kan^r = kanamycin resistance gene

CLONING AND EXPRESSION



Bacterial Expression Vectors

T7 Promoter System

The pT7-FLAG™ and pT7-MAT™ vectors offer the very strong T7/*lac* promoter. These expression vectors produce even higher yields of recombinant protein than the *tac* promoter system. However, the T7 promoter is known for background ("leaky") expression, which can be a drawback when recombinant proteins are toxic to the host cell. Therefore, Sigma's vectors contain the *lac* operator *(lacO)* sequences immediately downstream from the promoter to reduce leaky expression. Unlike the *tac* promoter system, pT7 vectors must be expressed in hosts containing a source of the T7 polymerase such as (DE3) lysogenic strains.

The MAT™ tag or Metal Affinity Tag (HNHRHKH) has been created for purification of recombinant MAT fusion proteins using HIS-Select™ Nickel and Cobalt Affinity Gels. HIS-Select products allow for highly selective purification of histidine-tagged fusion proteins such as MAT fusions. Many of our newest vectors make use of the MAT tag, often in combination with the well-known FLAG® tag. MAT tag containing vectors are offered in formats for N-terminal or C-terminal tagging.

pT7-FLAG-1 (4.9 kb)

pT7-FLAG™-1

Cytoplasmic expression of N-terminal Met-FLAG fusion proteins under the T7/*lac* promoter. Supplied with a pT7-FLAG-1-BAP control vector.

Product Code	Description	Size
P 1118	pT7-FLAG-1 Expression Vector	10 µg

pT7-FLAG-2 (5.1 kb)

pT7-FLAG™-2

Cytoplasmic expression of C-terminal FLAG fusion proteins under the T7/*lac* promoter. Supplied with a pT7-FLAG-2-BAP control vector.

Product Code	Description	Size
P 1243	pT7-FLAG-2 Expression Vector	10 µg

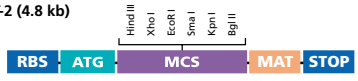
pT7-MAT-1 (4.8 kb)

pT7-MAT™-1

Cytoplasmic expression of N-terminal MAT fusion proteins under the T7/*lac* promoter. Supplied with a pT7-MAT-2+BAP Control Vector.

Product Code	Description	Size
E 5780	pT7-MAT-1 Expression Vector	10 µg

pT7-MAT-2 (4.8 kb)

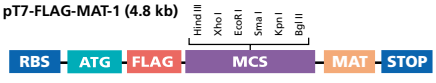


pT7-MAT™-2

Cytoplasmic expression of C-terminal MAT fusion proteins under the T7/*lac* promoter. Supplied with a pT7-MAT-2+BAP Control Vector.

Product Code	Description	Size
E 5655	pT7-MAT-2 Expression Vector	10 µg

pT7-FLAG-MAT-1 (4.8 kb)



pT7-FLAG-MAT™-1

Cytoplasmic expression of N-terminal Met-FLAG, C-terminal MAT dual tagged fusion proteins under the T7/*lac* promoter. Supplied with a pT7-FLAG-MAT-1+BAP Control Vector.

Product Code	Description	Size
E 5280	pT7-FLAG-MAT-1 Expression Vector	10 µg

pT7-MAT-FLAG-2 (4.8 kb)

pT7-MAT-FLAG™-2

Cytoplasmic expression of N-terminal MAT, C-terminal FLAG dual tagged fusion proteins under the T7/*lac* promoter. Supplied with a pT7-FLAG-MAT-1+BAP Control Vector.

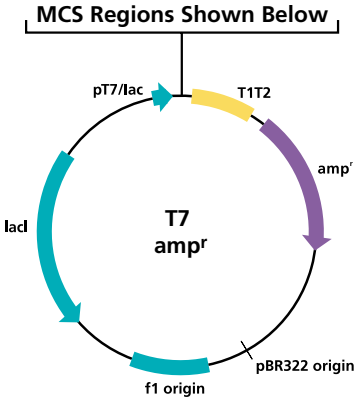
Product Code	Description	Size
E 4905	pT7-MAT-FLAG-2 Expression Vector	10 µg

CLONING AND EXPRESSION

Bacterial Expression Vectors

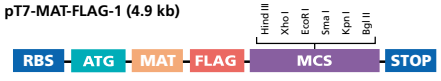
T7 Promoter System Continued

The recognition sequence for enterokinase, Asp-Asp-Asp-Asp-Lys, is found at the C-terminal end of the FLAG® epitope tag. Removal of FLAG is possible in all fusion proteins containing an N-terminal FLAG sequence. Dual tag fusion proteins may also be cleaved with enterokinase for removal of one or more tags, depending on the position of FLAG in the protein sequence.



pT7-MAT-FLAG™-1

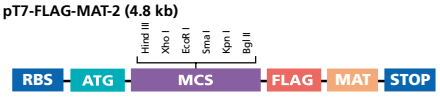
Cytoplasmic expression of N-terminal MAT-FLAG dual tagged fusion proteins under the T7/*lac* promoter. Supplied with a pT7-FLAG-MAT-1+BAP Control Vector.



Product Code	Description	Size
E 5155	pT7-MAT-FLAG-1 Expression Vector	10 µg

pT7-FLAG-MAT™-2

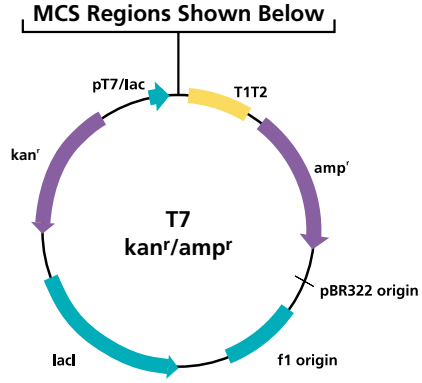
Cytoplasmic expression of C-terminal FLAG-MAT dual tagged fusion proteins under the T7/*lac* promoter. Supplied with a pT7-FLAG-MAT-1+BAP Control Vector.



Product Code	Description	Size
E 5030	pT7-FLAG-MAT-2 Expression Vector	10 µg

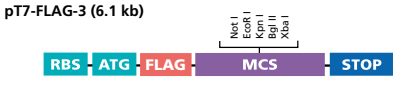
T7 Promoter System Kanamycin Resistance

The ampicillin and kanamycin antibiotic resistance genes are both present in pT7-FLAG-3 and pT7-FLAG-4 for selection flexibility in *E. coli*.



pT7-FLAG™-3

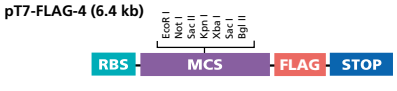
Cytoplasmic expression of N-terminal Met-FLAG fusion proteins under the T7/*lac* promoter. The presence of both the kanamycin resistance gene and the ampicillin resistance gene offers higher flexibility of selection. Supplied with a pT7-FLAG-3-BAP Control Vector.



Product Code	Description	Size
P 9618	pT7-FLAG-3 Expression Vector	10 µg

pT7-FLAG™-4

Cytoplasmic expression of C-terminal FLAG fusion proteins under the T7/*lac* promoter. The presence of both the kanamycin resistance gene and the ampicillin resistance gene offers higher flexibility of selection. Supplied with a pT7-FLAG-3-BAP Control Vector.



Product Code	Description	Size
P 9743	pT7-FLAG-4 Expression Vector	10 µg

CLONING AND EXPRESSION

E. coli Amino-Terminal FLAG® Expression Kit

COMPONENTS

Components
ANTI-FLAG® M1 monoclonal antibody
ANTI-FLAG® M2 affinity gel
ANTI-FLAG® M2 monoclonal antibody
Enterokinase
FLAG® peptide
pFLAG-ATS–BAP™ control plasmid
pFLAG–ATS™ expression vector (*OmpA* leader)
pFLAG–MAC™ expression vector (no leader)
C-24 sequencing primer
N-26 sequencing primer
N-terminal FLAG–BAP™ control protein

The *E. coli* Amino-Terminal FLAG® Expression Kit provides the specialized FLAG components needed to perform expression of a recombinant N-terminal FLAG fusion in *Escherichia coli*. The pFLAG-ATS™ (E 5769) and pFLAG-MAC™ (E 5644) Expression Vectors provide a choice of secreted or cytoplasmic expression of N-terminal FLAG fusion proteins, respectively, under control of the *tac* promoter. Also provided in the kit are controls for expression, detection, affinity purification and enterokinase removal of N-terminal FLAG.

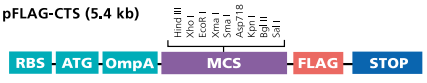
Product Code	Description	Size
FL-A	*E. coli* Amino-Terminal FLAG® Expression Kit	1 kit

E. coli Carboxy-Terminal FLAG® Expression Kit

COMPONENTS

Components
ANTI-FLAG® M2 monoclonal antibody
FLAG® peptide
pFLAG-CTC™ expression vector (no leader)
pFLAG-CTS-BAP™ control plasmid
pFLAG-CTS™ expression vector (*OmpA* leader)
N-26 sequencing primer
C-24 sequencing primers
C-terminal FLAG-BAP™ control protein

The *E. coli* Carboxy-Terminal FLAG® Expression Kit provides the specialized FLAG components needed to perform expression of a recombinant C-terminal FLAG fusion in *Escherichia coli*. The pFLAG-CTS™ (E 5269) and pFLAG-CTC™ (E 5394) Expression Vectors provide a choice of secreted or cytoplasmic expression of C-terminal FLAG fusion proteins, respectively, under control of the *tac* promoter. Also provided in the kit are controls for expression, detection, and affinity purification.



Product Code	Description	Size
FL-C	*E. coli* Carboxy-Terminal FLAG® Expression Kit	1 kit

E. coli FLAG-Shift™ Expression Kit

COMPONENTS

Components
ANTI-FLAG® M1 monoclonal antibody
ANTI-FLAG® M2 affinity gel
ANTI-FLAG® M2 monoclonal antibody
Enterokinase
FLAG® peptide
FLAG-Shift™$_{12}$–BAP control plasmid
FLAG–Shift™$_{12c}$ expression vector (no leader)
FLAG–Shift™$_{12}$ expression vector (*OmpA* leader
C-24 sequencing primer
N-26 sequencing primer
N-terminal FLAG–BAP™ control protein

The *E. coli* FLAG-Shift™ Expression Kit provides the specialized FLAG components needed for expression of an open reading frame (ORF) in all three reading frames as N-terminal FLAG fusion proteins in *Escherichia coli*. The pFLAG-Shift$_{12}$™ (E 8023) and pFLAG- Shift$_{12c}$™ (E 7898) Expression Vectors provide a choice of secreted or cytoplasmic expression of N-terminal FLAG fusion proteins, respectively, under control of the *tac* promoter. Both vectors contain 12 thymidine residues just upstream from the MCS region that result in frame-shifting during translation. Therefore, simultaneous expression of all three reading frames occurs. This feature is useful when the ORF is unknown as in genomic, cDNA or shotgun libraries. Also provided in the kit are controls for expression, detection, affinity purification and enterokinase removal of N-terminal FLAG.

Product Code	Description	Size
FL-S	*E. coli* FLAG-Shift™ Expression Kit	1 kit