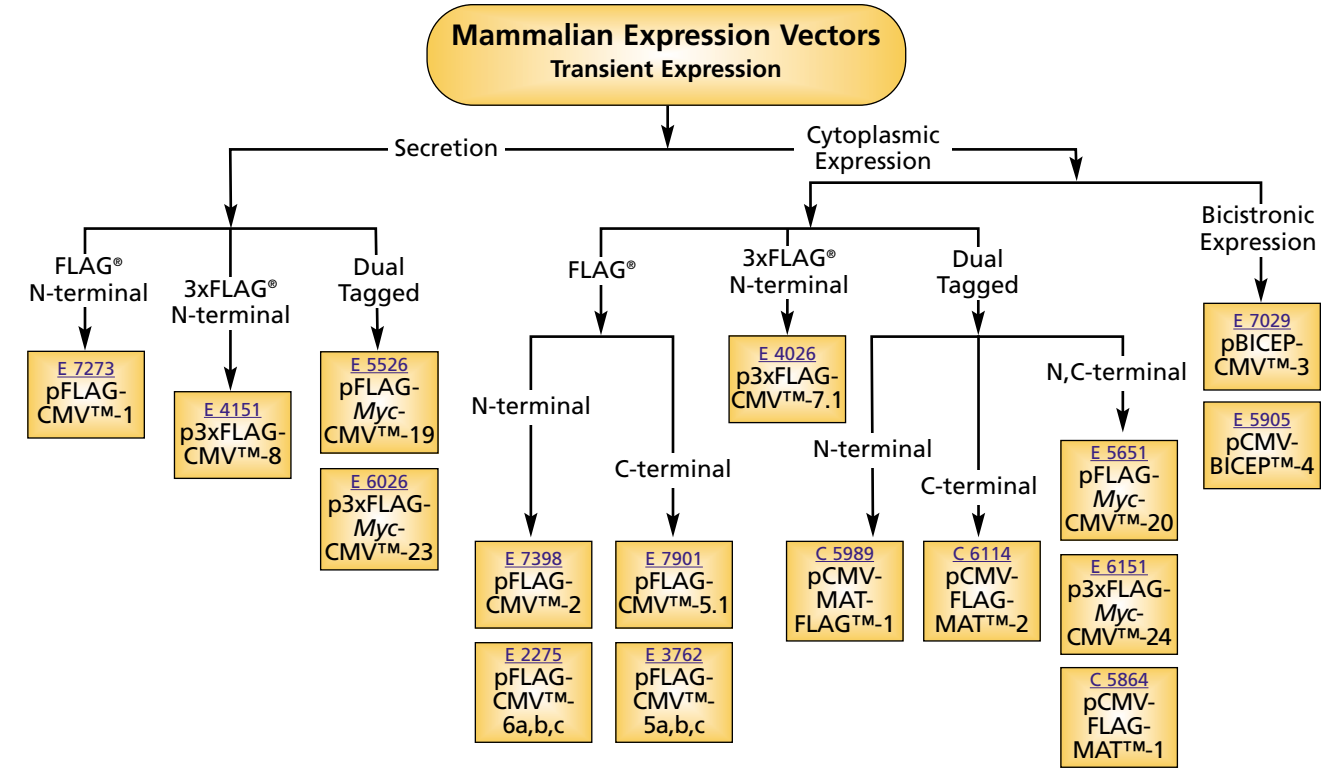
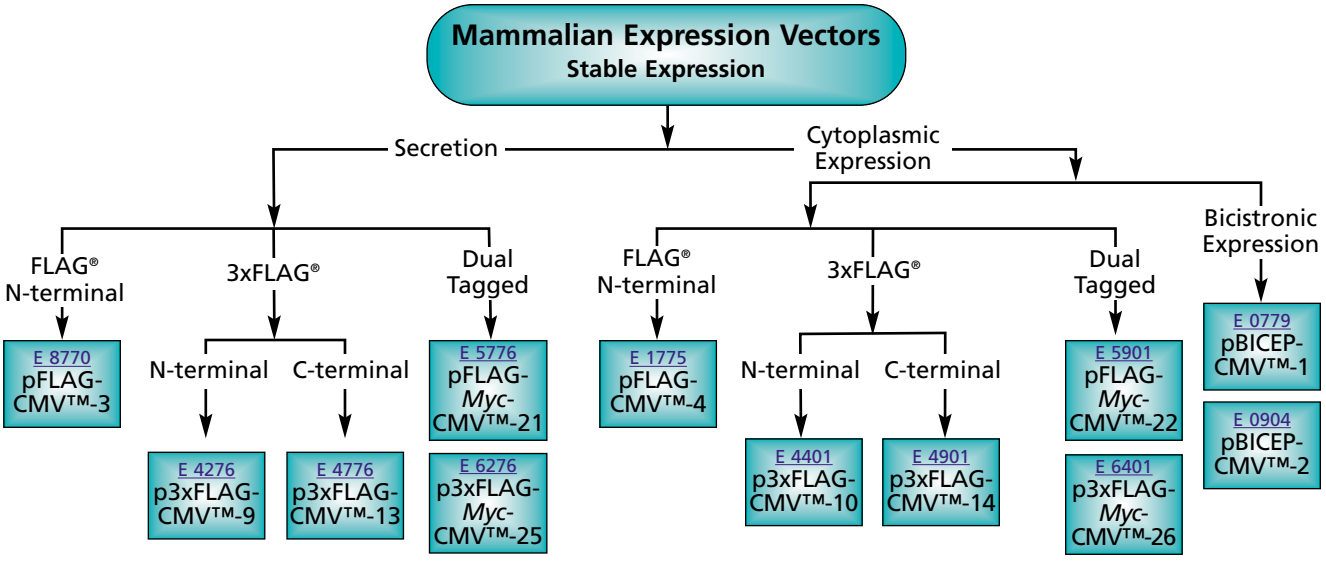
CLONING AND EXPRESSION

Mammalian Expression Vectors

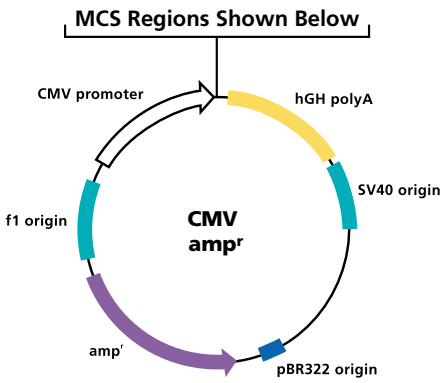
Mammalian FLAG® expression vectors produce a variety of FLAG fusion proteins that allow easy detection, purification and analysis of recombinant protein for a wide range of applications. Sigma's offering of CMV promoter-based vectors provides flexibility in transient or stable expression, cytoplasmic expression or secretion, and N- or C-terminal tagging with FLAG or 3xFLAG®. A number of dual tag vectors contain the c-*myc* epitope tag or the MAT™ (Metal Affinity Tag) in addition to FLAG or 3xFLAG.

The strong human cytomegalovirus (CMV) promoter regulatory region drives constitutive protein expression levels as high as 1 mg/L in COS cells. For less potent cell lines, protein levels are typically ~0.1 mg/L. The presence of the SV40 replication origin will result in high levels of DNA replication in SV40 replication permissive COS cells. CMV vectors contain the pMB1 (derivative of pBR322) origin for replication in bacterial cells, the β-lactamase gene for ampicillin resistance selection in bacteria, hGH polyA, and the f1 origin. Vectors containing the preprotrypsin leader (PPT) sequence direct secretion of FLAG fusion proteins into the culture medium for purification using ANTI-FLAG® antibodies, resins, and plates.





CLONING AND EXPRESSION



Mammalian Expression Vectors

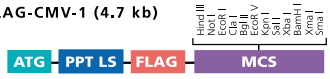
Transient Expression

CMV vectors for high-level transient expression offer a number of fusion tag formats (standard FLAG® and 3xFLAG® tags as well as dual tags such as FLAG-*Myc*, 3xFLAG-*Myc*, and FLAG-MAT™) as shown below.

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.

CMV vectors contain the pMB1 (derivative of pBR322) origin for replication in bacterial cells, the β-lactamase gene for ampicillin resistance selection in bacteria, hGH polyA, and the f1 origin. Vectors containing the preprotrypsin leader (PPT) sequence direct secretion of FLAG fusion proteins into the culture medium for purification using ANTI-FLAG® antibodies, resins, and plates.

pFLAG-CMV-1 (4.7 kb)

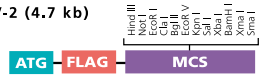


pFLAG-CMV™-1

For transient expression with extracellular secretion of N-terminal FLAG fusion proteins. Supplied with a pFLAG-CMV-1-BAP Control Vector.

Product Code	Description	Size
E 7273	pFLAG-CMV-1 Expression Vector	20 µg

pFLAG-CMV-2 (4.7 kb)



pFLAG-CMV™-2

For transient, cytoplasmic expression of N-terminal Met-FLAG fusion proteins. Supplied with a pFLAG-CMV-2-BAP Control Vector.

Product Code	Description	Size
E 7398	pFLAG-CMV-2 Expression Vector	20 µg

pFLAG-CMV-5.1 (4.7 kb)

pFLAG-CMV™-5.1

For transient, cytoplasmic expression of C-terminal FLAG fusion proteins. Supplied with a pFLAG-CMV-5b-BAP Control Vector.

Product Code	Description	Size
E 7901	pFLAG-CMV-5.1 Expression Vector	20 µg

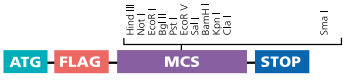
pFLAG-CMV-5a,b,c (4.7 kb)

pFLAG-CMV™-5a,b,c

For transient, cytoplasmic expression of C-terminal FLAG fusion proteins. The a,b,c series makes all three reading frames available for every restriction site in the MCS. Set includes 20 µg of each vector and pFLAG-CMV-5b-BAP control vector.

Product Code	Description	Size
E 3762	pFLAG-CMV-5a,b,c Expression Vectors	1 set

pFLAG-CMV-6a,b,c (4.7 kb)



pFLAG-CMV™-6a,b,c

For transient, cytoplasmic expression of N-terminal Met-FLAG fusion proteins. The a,b,c series makes all three reading frames available for every restriction site in the MCS. Set includes 20 µg of each vector and pFLAG-CMV-6a-BAP control vector.

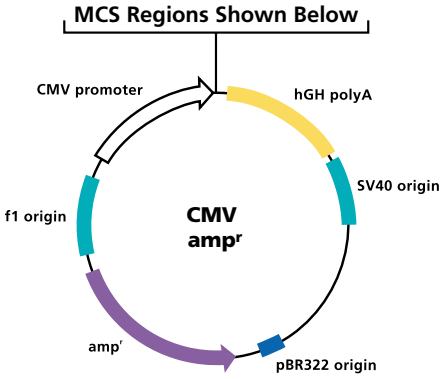
Product Code	Description	Size
E 2275	pFLAG-CMV-6a,b,c Expression Vectors	1 set

CLONING AND EXPRESSION

Mammalian Expression Vectors

Transient Expression Continued

The 3xFLAG® epitope tag is 20-200 times more sensitive than the original FLAG® tag. In cases of low-level expression, 3xFLAG is ideal. 3xFLAG is only 22 amino acids and is therefore unlikely to alter protein function or block other binding sites or epitopes. Like the original FLAG tag, 3xFLAG is very hydrophilic and can be cleaved with enterokinase.



p3xFLAG-CMV™-7.1

For transient, cytoplasmic expression of N-terminal Met-3xFLAG fusion proteins. Supplied with a p3xFLAG-CMV-7-BAP Control Vector.

Product Code	Description	Size
E 4026	p3xFLAG-CMV-7.1 Expression Vector	20 µg

p3xFLAG-CMV™-8

For transient expression and secretion of N-terminal 3xFLAG fusion proteins. Supplied with a p3xFLAG-CMV-7-BAP Control Vector.

Product Code	Description	Size
E 4151	p3xFLAG-CMV-8 Expression Vector	20 µg

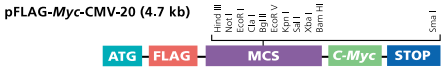
pFLAG-*Myc*-CMV™-19

For transient expression and secretion of dual tagged (N-terminal FLAG, C-terminal c-*myc*) fusion proteins. Supplied with a pFLAG-CMV-1-BAP Control Vector.

Product Code	Description	Size
E 5526	pFLAG-*Myc*-CMV-19 Expression Vector	20 µg

pFLAG-*Myc*-CMV™-20

For transient, cytoplasmic expression of dual tagged (N-terminal Met-FLAG, C-terminal c-*myc*) fusion proteins. Supplied with a pFLAG-CMV-2-BAP Control Vector.



Product Code	Description	Size
E 5651	pFLAG-*Myc*-CMV-20 Expression Vector	20 µg

p3xFLAG-*Myc*-CMV™-23

For transient expression and secretion of dual tagged (N-terminal 3xFLAG, C-terminal c-*myc*) fusion proteins. Supplied with a p3xFLAG-CMV-7-BAP Control Vector.

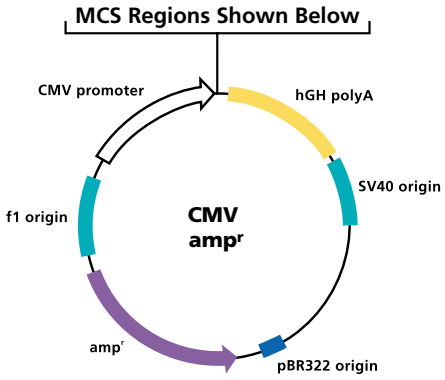
Product Code	Description	Size
E 6026	p3xFLAG-*Myc*-CMV-23 Expression Vector	20 µg

p3xFLAG-*Myc*-CMV™-24

For transient, cytoplasmic expression of dual tagged (N-terminal Met-3xFLAG, C-terminal c-*myc*) fusion proteins. Supplied with a p3xFLAG-CMV-7-BAP Control Vector.

Product Code	Description	Size
E 6151	p3xFLAG-*Myc*-CMV-24 Expression Vector	20 µg

CLONING AND EXPRESSION



Mammalian Expression Vectors

Transient Expression 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.

pCMV-FLAG-MAT™-1

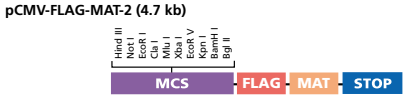
Transient cytoplasmic expression of N-terminal Met-FLAG, C-terminal MAT dual tagged fusion proteins under the CMV promoter. Supplied with pCMV-FLAG-MAT-1+MAPK1 Control Vector.

Product Code	Description	Size
C 5864	pCMV-FLAG-MAT-1 Expression Vector	20 µg

pCMV-MAT-FLAG™-1

Transient cytoplasmic expression of N-terminal MAT-FLAG dual tagged fusion proteins under the CMV promoter. Supplied with pCMV-FLAG-MAT-1+MAPK1 Control Vector.

Product Code	Description	Size
C 5989	pCMV-MAT-FLAG-1 Expression Vector	20 µg



pCMV-FLAG-MAT™-2

Transient cytoplasmic expression of C-terminal FLAG-MAT dual tagged fusion proteins under the CMV promoter. Supplied with pCMV-FLAG-MAT-1+MAPK1 Control Vector.

Product Code	Description	Size
C 6114	pCMV-FLAG-MAT-2 Expression Vector	20 µg

Mammalian Expression Vector Selection Table

Transient Expression *Note: BICEP™ vectors can be found on p. 39-40.*

Product	Product Name	PPT	FLAG	3x FLAG	*c-myc*	MAT	Ek Site	amp^r
E 7273	pFLAG-CMV-1	√	N				√	√
E 7398	pFLAG-CMV-2		N				√	√
E 7901	pFLAG-CMV-5.1		C					√
E 3762	pFLAG-CMV-5a,b,c		C					√
E 2275	pFLAG-CMV-6a,b,c		N				√	√
E 4026	p3xFLAG-CMV-7.1			N			√	√
E 4151	p3xFLAG-CMV-8	√		N			√	√
E 5526	pFLAG-*Myc*-CMV-19	√	N		C		√	√
E 5651	pFLAG-*Myc*-CMV-20		N		C		√	√
E 6026	p3xFLAG-*Myc*-CMV-23	√		N	C		√	√
E 6151	p3xFLAG-*Myc*-CMV-24			N	C		√	√
C 5864	pCMV-FLAG-MAT-1		N			C	√	√
C 5989	pCMV-MAT-FLAG-1		N			N	√	√
C 6114	pCMV-FLAG-MAT-2		C			C		√
E 7029	pBICEP-CMV-3		N (MCS1)				√	√
E 5905	pCMV-BICEP-4		N (MCS1)		N (MCS2)		√	√

N = N-terminal tag C = C-terminal tag PPT = preprotrypsin leader for direct secretion
c-*myc* = c-*myc* epitope MAT = metal affinity tag amp^r = ampicillin resistance gene
Ek = Enterokinase cleavage site (cleavage of dual-tagged proteins may result in removal of one or more tags)

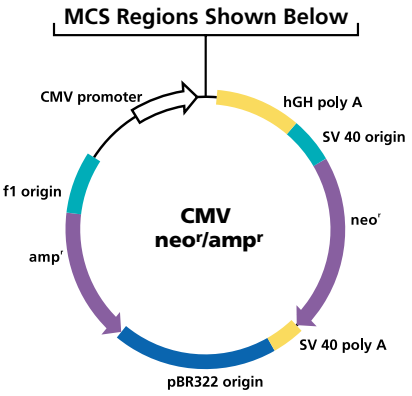
CLONING AND EXPRESSION

Mammalian Expression Vectors

Stable Expression

Our vectors for stable expression, as those for transient expression, contain the strong CMV promoter for high-level constitutive expression in mammalian cells. In addition, these constructs carry the aminoglycoside phosphotransferase II gene (neomycin resistance gene or neo^r) that confers resistance to aminoglycosides such as G 418 sulfate, allowing selection of stable transfectants.

CMV vectors also contain the pMB1 (derivative of pBR322) origin for replication in bacterial cells, the β-lactamase gene for ampicillin resistance selection in bacteria, hGH polyA, and the f1 origin. Vectors containing the preprotrypsin leader (PPT) sequence direct secretion of FLAG fusion proteins into the culture medium for purification using ANTI-FLAG® antibodies, resins, and plates.



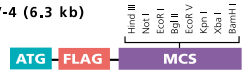
pFLAG-CMV™-3

For stable expression with extracellular secretion of N-terminal FLAG fusion proteins. Stable expression is accomplished by neomycin selection (G 418 sulfate). Supplied with a pFLAG-CMV-3-BAP Control Vector.

Product Code	Description	Size
E 8770	pFLAG-CMV-3 Expression Vector	20 µg

pFLAG-CMV™-4

For stable, cytoplasmic expression of N-terminal Met-FLAG fusion proteins. Stable expression is accomplished by neomycin selection (G 418 sulfate). Supplied with a pFLAG-CMV-4-BAP Control Vector.



Product Code	Description	Size
E 1775	pFLAG-CMV-4 Expression Vector	20 µg

p3xFLAG-CMV™-9

For stable expression with extracellular secretion of N-terminal 3xFLAG fusion proteins. Stable expression is accomplished by neomycin selection (G 418 sulfate). Supplied with a p3xFLAG-CMV-7-BAP Control Vector.

Product Code	Description	Size
E 4276	p3xFLAG-CMV-9 Expression Vector	20 µg

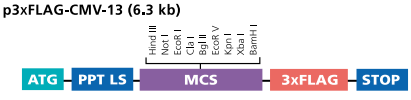
p3xFLAG-CMV™-10

For stable, cytoplasmic expression of N-terminal Met-3xFLAG fusion proteins. Stable expression is accomplished by neomycin selection (G 418 sulfate). Supplied with a p3xFLAG-CMV-7-BAP Control Vector.

Product Code	Description	Size
E 4401	p3xFLAG-CMV-10 Expression Vector	20 µg

p3xFLAG-CMV™-13

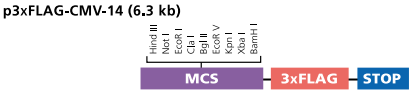
For stable expression with extracellular secretion of C-terminal 3xFLAG fusion proteins. Stable expression is accomplished by neomycin selection (G 418 sulfate). Supplied with a p3xFLAG-CMV-7-BAP Control Vector.



Product Code	Description	Size
E 4776	p3xFLAG-CMV-13 Expression Vector	20 µg

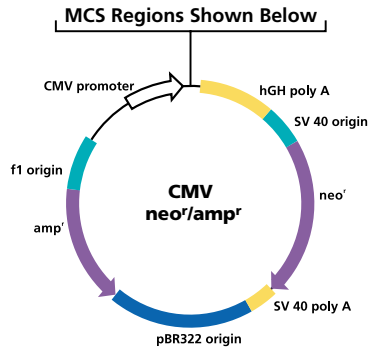
p3xFLAG-CMV™-14

For stable, cytoplasmic expression of C-terminal 3xFLAG fusion proteins. Stable expression is accomplished by neomycin selection (G 418 sulfate). Supplied with a p3xFLAG-CMV-7-BAP Control Vector.



Product Code	Description	Size
E 4901	p3xFLAG-CMV-14 Expression Vector	20 µg

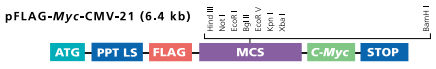
CLONING AND EXPRESSION



Mammalian Expression Vectors

Stable Expression Continued

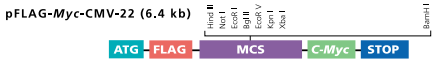
Vectors for stable expression of dual-tagged (FLAG®-*Myc* and 3xFLAG®-*Myc*) fusion proteins are shown below. Dual-tagged fusions provide flexibility in detection as well as a method to screen for full-length recombinant protein. 3xFLAG provides increased sensitivity of detection and is optimal for cases of low-level expression. 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.



pFLAG-*Myc*-CMV™-21

For stable expression with extracellular secretion of dual tagged (N-terminal FLAG, C-terminal c-*myc*) fusion proteins. Stable expression is accomplished by neomycin selection (G 418 sulfate). Supplied with a pFLAG-CMV-3-BAP Control Vector.

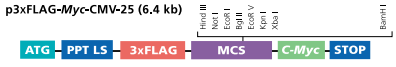
Product Code	Description	Size
E 5776	pFLAG-*Myc*-CMV-21 Expression Vector	20 µg



pFLAG-*Myc*-CMV™-22

For stable, cytoplasmic expression of dual tagged (N-terminal Met-FLAG, C-terminal c-*myc*) fusion proteins. Stable expression is accomplished by neomycin selection (G 418 sulfate). Supplied with a pFLAG-CMV-4-BAP Control Vector.

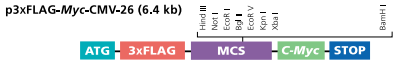
Product Code	Description	Size
E 5901	pFLAG-*Myc*-CMV-22 Expression Vector	20 µg



p3xFLAG-*Myc*-CMV™-25

For stable expression with extracellular secretion of dual tagged (N-terminal 3xFLAG, C-terminal c-*myc*) fusion proteins. Stable expression is accomplished by neomycin selection (G 418 sulfate). Supplied with a p3xFLAG-CMV-7-BAP Control Vector.

Product Code	Description	Size
E 6276	p3xFLAG-*Myc*-CMV-25 Expression Vector	20 µg



p3xFLAG-*Myc*-CMV™-26

For stable, cytoplasmic expression of dual tagged (N-terminal Met-3xFLAG, C-terminal c-*myc*) fusion proteins. Stable expression is accomplished by neomycin selection (G 418 sulfate). Supplied with a p3xFLAG-CMV-7-BAP Control Vector.

Product Code	Description	Size
E 6401	p3xFLAG-*Myc*-CMV-26 Expression Vector	20 µg

Mammalian Expression Vector Selection Table

Stable Expression

Note: BICEP vectors can be found on p. 39-40.

Product	Product Name	PPT	FLAG®	3x FLAG®	c-myc	Ek Site	amp^r	neo^r
E 8770	pFLAG-CMV-3	√	N			√	√	√
E 1775	pFLAG-CMV-4		N			√	√	√
E 4276	p3xFLAG-CMV-9	√		N		√	√	√
E 4401	p3xFLAG-CMV-10			N		√	√	√
E 4776	p3xFLAG-CMV-13	√		C			√	√
E 4901	p3xFLAG-CMV-14			C			√	√
E 5776	pFLAG-*Myc*-CMV-21	√	N		C	√	√	√
E 5901	pFLAG-*Myc*-CMV-22		N		C	√	√	√
E 6276	p3xFLAG-*Myc*-CMV-25	√		N	C	√	√	√
E 6401	p3xFLAG-*Myc*-CMV-26			N	C	√	√	√
E 0779	pBICEP-CMV-1		N (MCS1)			√	√	√
E 0904	pBICEP-CMV-2			N (MCS1)		√	√	√

N = N-terminal tag C = C-terminal tag neo^r = neomycin resistance for stable selection
PPT = preprotrypsin leader for direct secretion c-*myc* = c-*myc* epitope amp^r = ampicillin resistance gene
Ek = Enterokinase cleavage site (cleavage of dual-tagged proteins may result in removal of one or more tags)

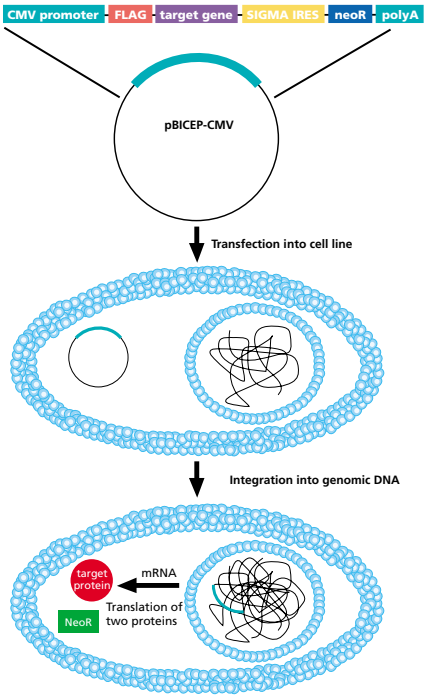
CLONING AND EXPRESSION

BICEP™ Expression Vectors

An optimized EMCV IRES offers the highest protein levels and stable expression.

BICEP (**BIC**istronic **E**xpression **P**lasmid) vectors contain an internal ribosomal entry site (IRES) element from the encephalomyocarditis virus (EMCV) for translation of two open reading frames (ORFs) from one bicistronic message. BICEP vectors are designed to drive transcription of the bicistronic message under control of the strong human cytomegalovirus (CMV) promoter regulatory region. IRESs are relatively short DNA sequences that can initiate RNA translation in a 5′ cap-independent fashion. Placement of the IRES and a second gene of interest (ORF 2) downstream of the first target gene (ORF 1) allows co-expression of ORF 1 in a cap-dependent manner and ORF 2 in a cap-independent fashion, thus facilitating translation of two proteins from one mRNA transcript. BICEP vectors permit co-expression of two genes of interest or co-expression of a target gene with a selection marker such as neo^r. Currently, pBICEP-CMV vectors for transient and stable mammalian expression are offered with various combinations of the FLAG®, 3xFLAG®, and c-*myc* epitope tags.

Sigma's EMCV IRES outperforms the competition by more than 30-fold as shown in Figure 1. pBICEP-CMV-1 and pBICEP-CMV-2 contain neomycin phosphotransferase (neo^r), which confers antibiotic resistance to G 418 sulfate (Figure 2). The neo^r is translationally regulated by the IRES. Mammalian cells that grow in the presence of G 418 are found to express high levels of recombinant protein due to both the target gene and selection marker being translated from one bicistronic message. BICEP vectors for stable expression also lack a mammalian origin of replication, and as a result, form stable cell lines through genomic integration of plasmid DNA. BICEP vectors outperform the competition due to strategic placement of the EMCV IRES to optimize intercistronic length and expression levels of recombinant protein.[1]



Stable Cells Retain High Levels of Expression

Features & Benefits

- Highest protein expression levels
- Stable cell line production without the concern of plasmid loss
- Maintains high expression levels after numerous passages
- Choices for stable or transient expression
- New vectors with two MCS regions for cloning your own stable selection marker or multiple genes of interest

Reference

1. Attal, J., Theron, M.C., Puissant, C., Houdebine, L.M., Medline® Effect of intercistronic length on internal ribosome entry site (IRES) efficiency in bicistronic mRNA. Gene Expression Volume 8, Issue 5-6, 1999, Pages 299-309.

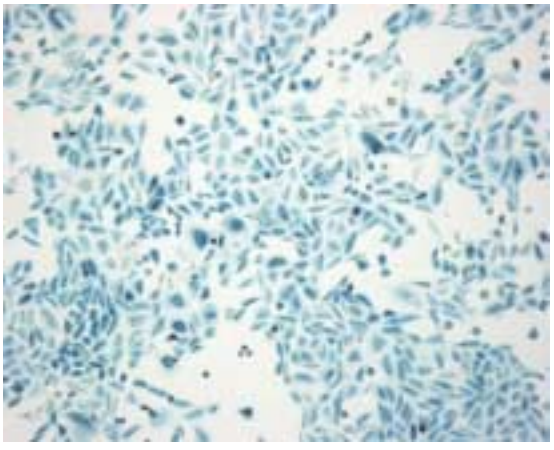


Figure 2. CHO Cells at Passage #20 stained for β-gal Expression

On day 1, CHO-K1 cells were seeded in a 6-well plate and were transfected with 2 µg/well of pBICEP-CMV-1+lacZ. Cells were grown in DMEM media supplemented with 10% FBS, 4 mM L-glutamine and 1 mg/ml G-418. At day 72, passage number reached 20. Cells were then fixed with 0.5% gluteraldehyde and stained using 0.25 mg/ml 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal).

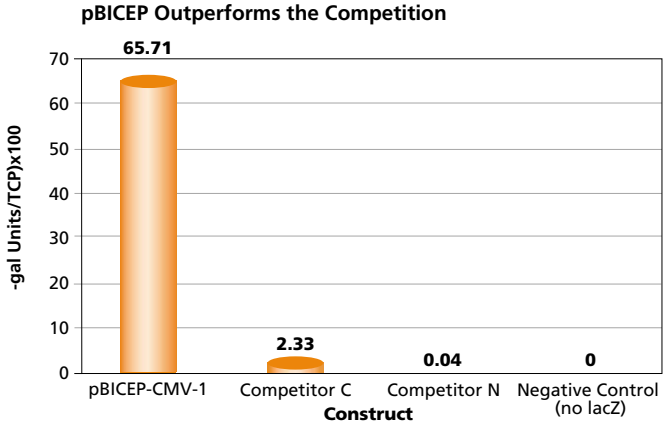
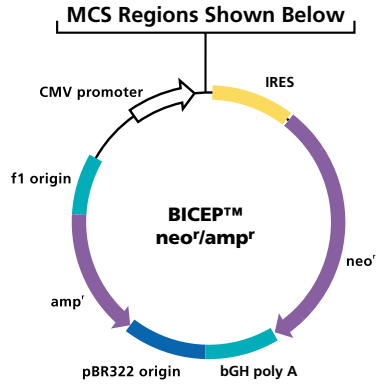


Figure 1.* β-*gal Assay Normalized to Total Cell Protein (TCP)

CHO-K1 cells were seeded in a 6-well plate. Cells were transfected with 2 µg/well of DNA using Sigma's Escort II reagent and grown in DMEM supplemented with 10% FBS plus 4 mM L-glutamine. G-418 was introduced 48 hrs post transfection at 1 mg/ml. During selection, β-gal and BCA assays were performed at day 28 using Sigma reagents.

CLONING AND EXPRESSION



BICEP™ Expression Vectors

Bicistronic Expression in Mammalian Systems

Stable Expression Vectors

pBICEP-CMV-1 and pBICEP-CMV-2 offer selection of cells expressing high levels of recombinant protein by expression of a target gene and the neo^r gene from a single bicistronic message. The neo^r gene is translated in a cap-independent fashion under control of the EMCV IRES. These vectors lack a mammalian origin of replication and thus likely form stable transfectants through genomic integration of plasmid DNA.

Product	Product Name	MCS1	neo^r
E 0779	pBICEP-CMV-1	FLAG (N)	√
E 0904	pBICEP-CMV-2	3x FLAG (N)	√

pBICEP-CMV™-1

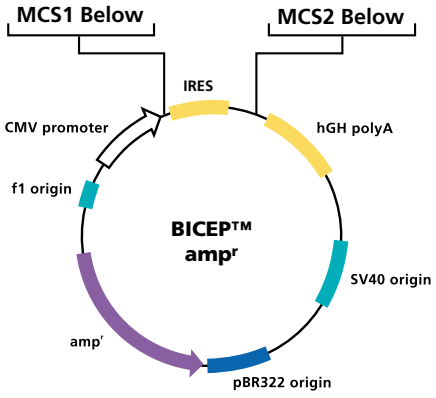
For stable, cytoplasmic expression of N-terminal Met-FLAG fusion proteins from bicistronic mRNA. Stable expression is accomplished by neomycin selection (G 418 sulfate). Supplied with a pBICEP-CMV-1+lacZ Control Vector.

Product Code	Description	Size
E 0779	pBICEP-CMV-1 Expression Vector	20 µg

pBICEP-CMV™-2

For stable, cytoplasmic expression of N-terminal Met-3xFLAG fusion proteins from bicistronic mRNA. Stable expression is accomplished by neomycin selection (G 418 sulfate). Supplied with a pBICEP-CMV-2+lacZ Control Vector.

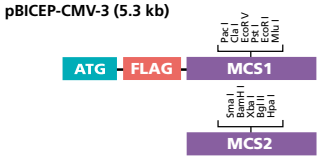
Product Code	Description	Size
E 0904	pBICEP-CMV-2 Expression Vector	20 µg



Transient Expression of Two Targets

pBICEP-CMV-3 and pCMV-BICEP-4 are mammalian bicistronic vectors for transient co-expression of any two genes of interest from one construct. Target genes are cloned into the two separate MCS regions. Genes cloned into MCS1 are expressed in a cap-dependent manner while genes cloned into MCS2 are translated in a cap-independent fashion under control of the EMCV IRES. Because both genes are expressed from one construct, it is likely that most transfected cells will express both genes unlike co-transfected populations. As a result, pBICEP-CMV-3 and pCMV-BICEP-4 are ideal for multi-subunit proteins, multi-protein complexes, and protein-protein interaction. These vectors contain the SV40 replication origin for episomal replication in appropriate host cells.

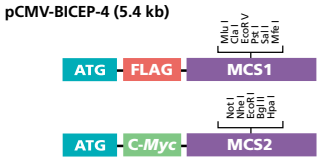
Product	Product Name	MCS1	MCS2
E 7029	pBICEP-CMV-3	FLAG (N)	untagged
E 5905	pCMV-BICEP-4	FLAG (N)	c-*myc* (N)



pBICEP-CMV™-3

For transient, cytoplasmic expression of an N-terminal Met-FLAG fusion and a second gene of interest from bicistronic mRNA. Two genes can be cloned into MCS1 and MCS2 for transcription of a single message from the CMV promoter.

Product Code	Description	Size
E 7029	pBICEP-CMV-3 Expression Vector	20 µg



pCMV-BICEP™-4

For transient, cytoplasmic expression of N-terminal Met-FLAG fusions and N-terminal c-*myc* fusions from bicistronic mRNA. Two genes can be cloned into MCS1 and MCS2 for transcription of a single message from the CMV promoter. Supplied with a control vector (pCMV-BICEP-4+p53/LTA) containing FLAG-p53 and c-*myc*-Large T antigen in MCS1 and MCS2.

Product Code	Description	Size
E 5905	pCMV-BICEP-4 Expression Vector	20 µg

CLONING AND EXPRESSION

Mammalian Amino-terminal FLAG® Transient Expression Kit

The Mammalian Amino-Terminal FLAG® Transient Expression Kit provides all of the specialized FLAG components needed to perform expression of a recombinant N-terminal FLAG fusion in mammalian cells. The pFLAG-CMV™-1 (E 7273) and pFLAG-CMV™-2 (E 7398) Expression Vectors allow transient expression of secreted or cytoplasmically expressed fusion proteins. Also provided in the kit are controls for expression, detection, and affinity purification.

Product Code	Description	Size
FL-MA	Mammalian Amino-Terminal FLAG® Transient Expression Kit	1 kit

COMPONENTS

Components
ANTI-FLAG® M2 monoclonal antibody
ANTI-FLAG® M5 monoclonal antibody
C-CMV-24 sequencing primer
N-CMV-30 sequencing primer
FLAG® peptide
Met-FLAG-BAP™ control protein
pFLAG-CMV™-1-BAP control plasmid
pFLAG-CMV™-2-BAP control plasmid
pFLAG-CMV™-2 expression vector (no leader)
pFLAG-CMV™-1 expression vector (preprotrypsin leader)

Mammalian Amino-terminal FLAG® Stable Expression Kit

The Mammalian Amino-Terminal FLAG® Stable Expression Kit provides the specialized FLAG components needed for expression of a recombinant N-terminal FLAG fusion in mammalian cells. The pFLAG-CMV™-3 (E 8770) and pFLAG-CMV™-4 (E 1775) Expression Vectors allow transient or stable expression of secreted or cytoplasmically expressed fusion proteins. Stable expression is accomplished by neomycin selection (G 418). Also provided in the kit are controls for expression, detection, and affinity purification.

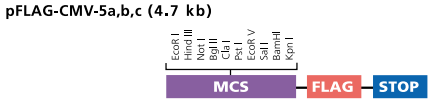
Product Code	Description	Size
FL-MA-S	Mammalian Amino-Terminal FLAG® Stable Expression Kit	1 kit

COMPONENTS

Components
ANTI-FLAG® M2 monoclonal antibody
ANTI-FLAG® M5 monoclonal antibody
C-CMV-24 sequencing primer
N-CMV-30 sequencing primer
FLAG® Peptide
Met-FLAG-BAP™ control protein
pFLAG-CMV™-3-BAP control plasmid
pFLAG-CMV™-4-BAP control plasmid
pFLAG-CMV™-4 expression vector (no leader)
pFLAG-CMV™-3 expression vector (preprotrysin leader)

Mammalian Carboxy-terminal FLAG® Transient Expression Kit

The Mammalian Carboxy-Terminal FLAG® Transient Expression Kit provides the specialized FLAG components needed for transient expression of a recombinant C-terminal FLAG fusion in mammalian cells. The pFLAG-CMV™-5a,b,c Expression Vectors (E 3762) make all reading frames of the MCS available at each restriction site for cloning of an open reading frame. All three vectors lack a translational start signal, so the start signal and any secretion leaders must be inserted along with the gene of interest. Also provided in the kit are controls for expression, detection, and affinity purification.



Product Code	Description	Size
FL-MC	Mammalian Carboxy-Terminal FLAG® Transient Expression Kit	1 kit

COMPONENTS

Components
ANTI-FLAG® M2 affinity gel
ANTI-FLAG® M2 monoclonal antibody
C-CMV-24 sequencing primer
N-CMV-30 sequencing primer
FLAG® peptide
pFLAG-CMV™-5b–BAP control plasmid
pFLAG-CMV™-5a expression vector
pFLAG-CMV™-5b expression vector
pFLAG-CMV™-5c expression vector
C-terminal FLAG–BAP™ control protein