

Product Information

pCMV-FLAG®-MAT-Tag®-1 Expression Vector

Catalog Number **C5864**

Storage Temperature -20 °C

TECHNICAL BULLETIN

pCMV-FLAG®-MAT-Tag®-1 is a 4711 bp mammalian expression vector used for the transient production of dual tagged fusion proteins in mammalian cells. This vector is a derivative of the pCMV5 transient expression vector¹ for intracellular expression of a properly inserted open reading frame as a Met-N-terminal FLAG, C-terminal MAT-Tag (Metal Affinity Tag) fusion protein. The promoter-regulatory region of the human cytomegalovirus² drives transcription of the fusion containing the FLAG epitope (DYKDDDDK)³ and the transition metal binding, e.g. Ni²⁺ and Co²⁺, MAT-Tag (HNHRHKH). The pCMV-FLAG-MAT-Tag-1 expression vector is a shuttle vector containing both bacterial and SV40 origins of replication for propagation in both *E. coli* and mammalian cells. Efficiency of replication in mammalian cells is optimal when using host cells that express the SV40 large T antigen (e.g. COS-7). Removal of the FLAG tag is possible using enterokinase which cleaves following the Asp-Asp-Asp-Asp-Lys recognition site at the C-terminal end of FLAG.

pCMV-FLAG-MAT-Tag-1 may be used in conjunction with the Director™ Universal PCR System, Catalog Number RDC1, for a simple, rapid, and universal method to directionally clone PCR products. The MCS has been optimized for use with the *Hind* III/*Bgl* II pair of 5' overhang producing restriction enzymes often used in the Director system. The FLAG epitope of the fusion protein may be detected using Monoclonal ANTI-FLAG M2, Catalog Number F3165, and the fusion protein may be purified using the ANTI-FLAG M2 Affinity Gel, Catalog Number A2220. The fusion protein may also be purified utilizing the metal affinity properties of the MAT-Tag by using HIS-Select® Nickel Affinity Gel, Catalog Number P6611. Sigma-Aldrich offers a wide selection of related ANTI-FLAG and HIS-Select products; please visit www.sigma-aldrich.com for a complete listing of antibody conjugates, resins, and affinity capture plates.

Reagents

- pCMV-FLAG-MAT-Tag-1 Expression Vector, Catalog Number E1155 20 µg
0.5 mg/ml in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.
- pCMV-FLAG-MAT-Tag-1-MAPK1 Control Vector, Catalog Number C0489 20 µg
0.5 mg/ml in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.

Precautions/Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage

Store at -20 °C.

Vector Features

The following table provides map positions of the features in the pCMV-FLAG-MAT-Tag-1 vector. Sequence verification of the MCS can be performed using N-CMV-30 Sequencing Primer, Catalog Number P5350, and C-CMV-24 Sequencing Primer, Catalog Number P5475.

| Feature | Map Position |
|---|--------------|
| CMV Promoter | 166-916 |
| N-CMV-30 Sequencing Primer Binding Site | 825-854 |
| FLAG | 935-958 |
| MCS | 956-1015 |
| MAT-Tag | 1016-1036 |
| hGH polyA | 1055-1674 |
| C-CMV-24 Sequencing Primer Binding Site | 1112-1135 |
| SV40 ori | 1693-2037 |
| pBR322 ori | 2940-3059 |
| Amp | 3206-4124 |
| f1 ori | 4259-4711 |

References

1. Andersson, S., et al., *J. Biol. Chem.*, **264**, 8222-8229 (1989).

2. Thomsen, D.R., et al., *Proc. Natl. Acad. Sci. USA*, **81**, 659-663 (1984)

3. Hopp, T.V., et al., *Bio/Technology*, **6**, 1204-1210 (1988).

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AH,PHC,MAM 04/11-1

pCMV-FLAG-MAT-1 (4.7 kb)

ATG FLAG MCS MAT STOP

Restriction sites: Hind III, Not I, EcoR I, Cla I, Xba I, EcoR V, Kpn I, BamH I, Bgl II

pCMV-FLAG-MAT-Tag-1 4711 bp

CMV promoter, hGH polyA, SV40 origin, pBR322 origin, amp^r, f1 origin

Multiple Cloning Site

FLAG Peptide Sequence

| | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Asp | Tyr | Lys | Asp | Asp | Asp | Asp | Lys |
| ATG | GAC | TAC | AAA | GAC | GAT | GAC | GAC | AAG |
| TAC | CTG | ATG | TTT | CTG | CTA | CTG | CTG | TTC |

Not I, EcoR I, Hind III

MAT Sequence

| | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|------|
| His | Asn | His | Arg | His | Lys | His | STOP |
| CAC | AAC | CAC | CGC | CAC | AAG | CAC | TAA |
| GTG | TTG | GTG | GCG | GTG | TTC | GTG | ATT |

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