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# **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 vector<sup>1</sup> 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 cytomegalovirus<sup>2</sup> drives transcription of the fusion containing the FLAG epitope (DYKDDDDK)<sup>3</sup> and the transition metal binding, e.g. Ni<sup>2+</sup> and Co<sup>2+</sup>, 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.sigmaaldrich.com for a complete listing of antibody conjugates, resins, and affinity capture plates.

#### Reagents

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

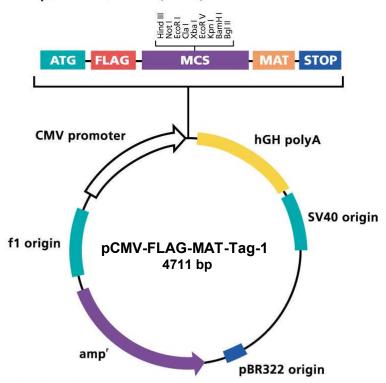
- Andersson, S., et al., J. Biol. Chem., 264, 8222-8229 (1989).
- Thomsen, D.R., et al., *Proc. Natl. Acad. Sci. USA*, 81, 659-663 (1984)
- 3. Hopp, T.V., et al., *Bio/Technology*, **6**, 1204-1210 (1988).

FLAG, MAT-Tag, and HIS-Select are registered trademarks of Sigma-Aldrich<sup>®</sup> Biotechnology LP and Sigma-Aldrich Co.

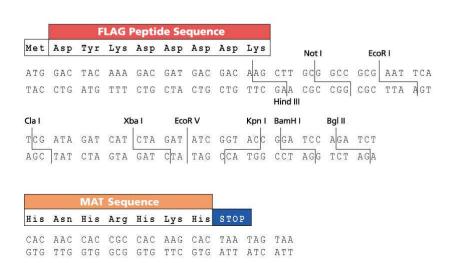
Director is a trademark of Sigma-Aldrich Biotechnology LP and Sigma-Aldrich Co.

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## pCMV-FLAG-MAT-1 (4.7 kb)



## **Multiple Cloning Site**



Sigma brand products are sold through Sigma-Aldrich, Inc.