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Product Information

pT7-FLAG®-MAT-Tag™-2 Expression Vector

Catalog Number **E5030**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

pT7-FLAG-MAT-Tag-2 is a 4839 bp *Escherichia coli* expression vector used for cytoplasmic expression of a properly inserted open reading frame as a dual tagged C-terminal FLAG-MAT-Tag (Metal Affinity Tag) fusion protein. The fusion contains the FLAG epitope (DYKDDDDK)[1] and the transition metal binding, e.g. Ni^{+2} and Co^{+2}, MAT-Tag (HNHRHKH). The promoter region of the very strong phage T7 promoter [2,3] drives transcription of ORF-FLAG-MAT-Tag fusion constructs. This vector requires the use of *E. coli* cells containing a source of the T7 RNA polymerase, such as BL21(DE3) cells. Transcription is regulated in these cells by having the T7 RNA polymerase gene under the control of the inducible *lacUV5* promoter. Tighter repression of basal level transcription is provided by the inclusion of *lacO* sequences immediately downstream of the pT7 promoter and having the *lac* repressor gene (*lacI)* on the plasmid. Removal of the MAT-Tag is possible using enterokinase which cleaves following the Asp-Asp-Asp-Asp-Lys recognition site at the C-terminal end of FLAG.

pT7-FLAG-MAT-Tag-2 may be used in conjunction with the Director™ Universal PCR System, Catalog Number RDC1 for a simple, rapid and universal method to directionally clone and express PCR products. The MCS has been optimized for use with the *Hind* III and *Bgl* II restriction enzymes often used in the Director system.

The C-terminal FLAG-MAT-Tag fusion protein may be detected using Monoclonal ANTI-FLAG M2, Catalog Number F3165, and purified using ANTI-FLAG M2 Affinity Gel, Catalog Number A 2220. Additionally, the fusion protein may be purified utilizing the metal affinity properties of the MAT tag by using HIS-Select® Nickel Affinity Gel, Catalog Number P 6611. 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 Provided

- pT7-FLAG-MAT-Tag-2 Expression Vector, 10 µg, Catalog Number E3155, 0.5 mg/ml in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.
- pT7–FLAG-MAT-Tag-1-BAP Control Vector, 1 µg, Catalog Number C7114, 0.05 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

Product ships on dry ice. Store at –20 °C.

Vector Features

The following table provides map positions to key features in the pT7-FLAG-MAT-Tag-2 vector. Sequence verification of the MCS can be performed using the C-24 Sequencing Primer, Catalog Number P7957. The sequence 5'-CTATCATGCCATACCGCGAAAGG-3', available from Sigma-Genosys, is recommended for sequencing through the N-terminal junction.

Feature	Map Position
Recommended 5' primer sequence binding site	31-53
T7 Promoter	72-91
lacO	92-111
Ribosomal Binding Site	143-148
MCS	159-194
FLAG epitope	195-218
MAT-Tag	222-242
C-24 Sequencing Primer Binding Site	268-291
T1/T2 terminator	299-669
beta-lactamase (amp^r)	768-1625
pBR322 ori	1833-1952
f1 ori	2616-3079
lacI	3757-4839

References

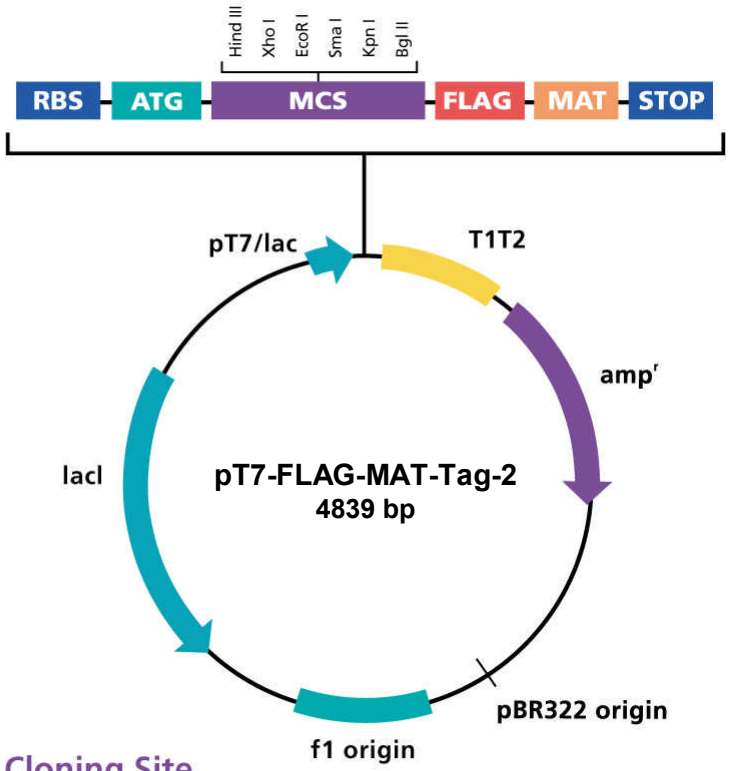
1. Hopp, T.V., et al., *Bio/Technology*, **6**, 1204-1210 (1988).
2. Moffet, B.A. et al., *Journal of Molecular Biology,* **189,** 113-130 (1986).
3. Rosenberg, A.H. et al., *Gene*, **56**, 125-135 (1987).

Academic and Non-Profit Laboratory Assurance Letter

The T7 system is based on technology developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy and is the subject of U.S. Patent No. 5,693,489 (expiration date, December 2, 2014) assigned to Brookhaven Science Associates, LLC. (BSA). BSA will grant a nonexclusive license for the use of this technology, including the enclosed material, based upon the following assurances:

1. These materials are to be used for noncommercial research purposes only. A separate license is required for any commercial use, including use of these materials for research purposes or production purposes by any commercial entity. Information about commercial licenses may be obtained from the Office of Technology Commercialization and Partnerships, Brookhaven National Laboratory, Bldg. 490-C, P.O. Box 5000, Upton, New York 11973-5000, telephone (631)-344-7134.
2. No materials that contain the cloned copy of T7 gene 1, the gene for T7 RNA polymerase, may be distributed further to third parties outside of your laboratory, unless the recipient receives a copy of this license and agrees to be bound by its terms. This limitation applies to strains of BL21(DE3), BL21(DE3)pLysS, and BL21(DE3)pLysE, and any derivatives.
3. You may refuse this license by returning the enclosed materials unused. By keeping or using the enclosed materials, you agree to be bound to the terms of this license."

AH,RS,PHC 11/10-1



Multiple Cloning Site

Met Hind III XhoI EcoR I Sma I Kpn I Bgl II

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ATG AAG CTT CCT CGA GTG AAT TCC CCG GGT ACC AGA TCT
TAC TTC GAA GGA GCT CAC TTA AGG GGC CCA TGG TCT AGA
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FLAG Peptide Sequence									MAT Sequence								
Asp	Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Ala	His	Asn	His	Arg	His	Lys	His	STOP	

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GAC TAC AAG GAC GAC GAT GAC AAG GCT CAC AAC CAC CGT CAC AAA CAC TGA
CTG ATG TTC CTG CTG CTA CTG TTC CGA GTG TTG GTG GCA GTG TTT GTG ACT
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