

Product Information

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

Catalog Number **E4905**

Storage Temperature -20 °C

TECHNICAL BULLETIN

Product Description

pT7-MAT-Tag-FLAG-2 is a 4838 bp *Escherichia coli* expression vector for cytoplasmic expression of a properly inserted open reading frame as a N-terminal MAT-Tag (Metal Affinity Tag), C-terminal FLAG fusion protein. The fusion contains the transition metal binding, e.g. Ni⁺² and Co⁺², MAT-Tag (HNHRHKH) and the FLAG epitope (DYKDDDDK)¹. The promoter region of the very strong phage T7 promoter^{2,3} drives transcription of MAT-Tag-ORF-FLAG 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.

pT7-MAT-Tag-FLAG-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 N-terminal MAT-Tag, C-terminal FLAG fusion protein may be detected using Monoclonal ANTI-FLAG M2, Catalog Number F3165, and purified using ANTI-FLAG M2 Affinity Gel, Catalog Number A2220. 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 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

- pT7-MAT-Tag-FLAG-2 Expression Vector, 10 µg, Catalog Number E3030, 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-MAT-Tag-FLAG-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
pT7 Promoter	72-91
<i>lacO</i>	92-111
Ribosomal Binding Site	143-148
MAT tag	161-181
MCS	182-217
FLAG epitope	218-241
C-24 Sequencing Primer Binding Site	267-290
T1/T2 terminator	298-668
beta-lactamase (amp ^r)	767-1624
pBR322 ori	1832-1956
f1 ori	2615-3078
<i>lacI</i>	3756-4838

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).

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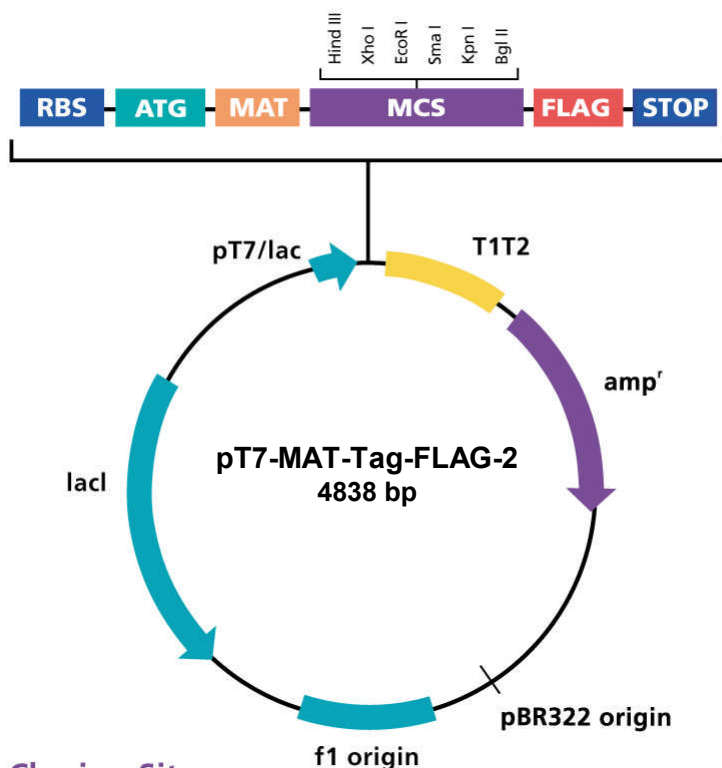
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Academic and Non-Profit Laboratory Assurance Letter

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:

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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.
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Multiple Cloning Site

MAT Sequence											
Met	Ala	His	Asn	His	Arg	His	Lys	His			
ATG	GCT	CAC	AAC	CAC	CGT	CAC	AAA	CAC	AAG	CTT	CCT
TAC	CGA	GTG	TTG	GTG	GCA	GTG	TTT	GTG	TTC	GAA	GGA

FLAG Peptide Sequence							
Asp	Tyr	Lys	Asp	Asp	Asp	Asp	Lys
GAC	TAC	AAG	GAC	GAC	GAT	GAC	AAG
CTG	ATG	TTC	CTG	CTG	CTA	CTG	TTC

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