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Product Information

pT7-MAT-Tag™-2 Expression Vector

Catalog Number **E5655**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

pT7-MAT-Tag-2 is a 4808 bp *Escherichia coli* expression vector used for cytoplasmic expression of a properly inserted open reading frame as a C-terminal MAT-Tag (Metal Affinity Tag) fusion protein. The MAT tag (HNHRHKH) is a transition metal binding, e.g. Ni^{+2} and Co^{+2}, sequence useful for high quality purification. The promoter region of the very strong phage T7 promoter[1,2] drives transcription of ORF-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.

pT7-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.

C-terminal MAT-Tagged fusion proteins 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 HIS-Select products. Please visit www.sigma-aldrich.com for a complete listing of resins and affinity capture plates.

Reagents

- pT7-MAT-Tag-2 Expression Vector, 10 µg, Catalog Number E4405, 0.5 mg/ml in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.
- pT7-MAT-Tag-2-BAP Control Vector, 1 µg, Catalog Number C7989, 0.05 mg/ml in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA

Precautions and Disclaimer

This product is for R&D use only. Not for drug, household or other uses. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

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-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	159-190
MAT-Tag	191-211
C-24 Sequencing Primer Binding Site	237-260
T1/T2 terminator	268-638
β-lactamase (amp^r)	737-1594
pBR322 ori	1802-1921
f1 ori	2585-3048
lacI	3726-4808

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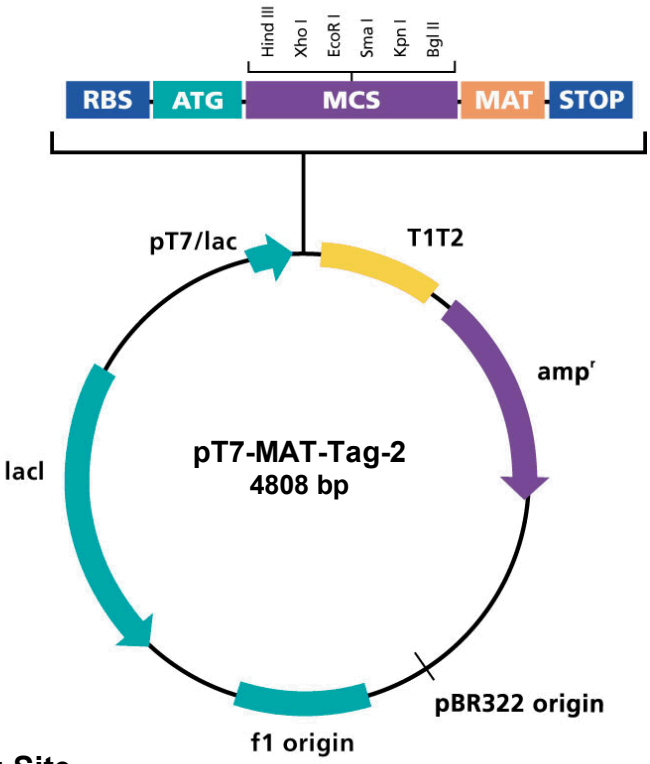
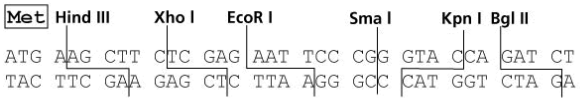
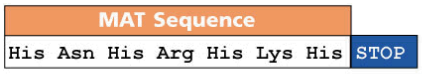
References

1. Rosenberg, A. H., et al., Vectors for selective expression of cloned DNAs by T7 RNA polymerase. *Gene,* **56**, 125-135 (1987).
2. Studier, F. W., and Moffatt, B. A., Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.,* **189,** 113-130 (1986).



Multiple Cloning Site



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CAC AAC CAC CGT CAC AAA CAC TGA
GTG TTG GTG GCA GTG TTT GTG ACT
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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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