3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

pT7-FLAG™-2 Expression Vector

Catalog Number **P1243**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

pT7-FLAG-2 is a 4815 bp *Escherichia coli* expression vector used for cloning and cytoplasmic expression of a properly inserted open reading frame as a C-terminal FLAG® fusion protein containing the FLAG epitope (DYKDDDDK). The promoter region of the very strong phage T7 promoter1,[2] drives transcription of ORF-FLAG fusion constructs. This vector requires the use of *E. coli* cells containing a source of the T7 polymerase, such as BL21(DE3)T1^{R} cells, Catalog Number B2935.

Transcription is regulated in these cells by having the T7 polymerase gene under the control of the *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.

The C-terminal FLAG fusion protein may be detected using Monoclonal ANTI-FLAG® M2, Catalog Number F3165, and purified using the ANTI-FLAG M2 Affinity Gel, Catalog Number A2220. Please visit www.sigma-aldrich.com for a complete listing of resins and affinity capture plates.

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

pT7-FLAG™-2 Features

Feature	Map Position
T7 Promoter	72-91
lacO	92-111
Recommended 5' primer sequence binding site	31-53
Ribosomal Binding Site	143-148
MCS	159-194
FLAG tag	195-218
C-24 Sequencing Primer Binding Site	244-267
T1/T2 terminator	275-645
β-lactamase (amp^{r})	744-1601
pBR322 ori	1809-1928
f1 ori	2592-3055
lacI	3733-4815

Reagents

- pT7-FLAG-2 Expression Vector, 10 µg
  Catalog Number P6867
  0.5 mg/ml in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.

- pT7-FLAG-2-BAP Control Vector 1 µg
  Catalog Number P7117
  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

This product ships on dry ice and storage at –20 °C is recommended.

References

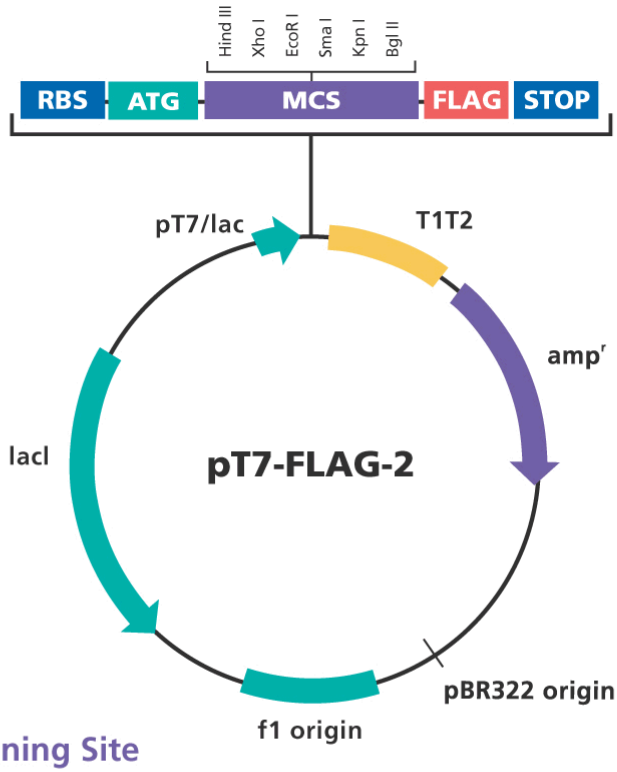
1. Rosenberg, A. H, et al., Vectors for selective expression of cloned DNAs by T7 RNA polymerase. *Gene*, **56**, 125-135 (1987).
2. Studier, F. W., and Moffatt, B. A., Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.,* **189,** 113-130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

Academic and Non-Profit Laboratory Assurance Letter

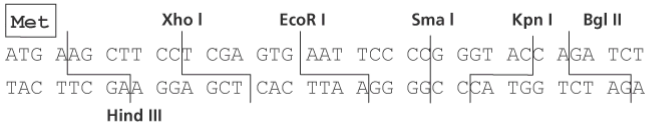
The T7 system is based on technology developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy and is the subject of U.S. Patent No. 5,693,489 (expiration date, December 2, 2014) assigned to Brookhaven Science Associates, LLC. (BSA). BSA will grant a nonexclusive license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

pT7-FLAG-2 (4.8 kb)



Multiple Cloning Site
(pT7-FLAG-2)



GAC TAC AAG GAC GAC GAT GAC AAG TGA
CTG ATG TTC CTG CTG CTA CTG TTC ACT

AH,PHC 11/10-1