

## Product Information

### pT7-FLAG™-4 Expression Vector

Catalog Number **P9743**

Storage Temperature  $-20\text{ }^{\circ}\text{C}$

## TECHNICAL BULLETIN

### Product Description

pT7-FLAG™-4 is a 6451 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 promoter<sup>1,2</sup> 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) cells, Catalog Numbers B2935 or B3310.

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 *amp<sup>r</sup>* and *kan<sup>r</sup>* antibiotic resistance genes are both present for selection flexibility in *E. coli*.

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](http://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-4 vector. Sequence verification of the MCS can be performed using the C-24 Sequencing Primer, Catalog Number P7957. The sequence 5'-GTAACATCAGAGATTTTGAGACAC-3', available from Sigma-Genosys, is recommended for sequencing through the N-terminal junction.

### pT7-FLAG-4 Features

Feature	Map Position
T7 Promoter	140-156
<i>lacO</i>	159-184
Recommended 5' primer sequence binding site	76-99
Ribosomal Binding Site	210-215
MCS	220-272
FLAG tag	276-299
C-24 Sequencing Primer Binding Site	323-346
T1/T2 terminator	354-724
$\beta$ -lactamase ( <i>amp<sup>r</sup></i> )	823-1680
pBR322 ori	1888-2007
f1 ori	2671-3134
<i>lacI</i>	3812-4894
APH-II ( <i>kan<sup>r</sup></i> )	5639-6451

### Reagents

- pT7-FLAG-4 Expression Vector 10  $\mu\text{g}$   
Catalog Number P8617  
0.5 mg/ml in 10 mM Tris-HCl, pH 8.0,  
1 mM EDTA
- pT7-FLAG-3-BAP Control Plasmid 1  $\mu\text{g}$   
Catalog Number P8242  
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. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

**Storage/Stability**

This product ships on dry ice. Store at  $-20^{\circ}\text{C}$ .

pT7-FLAG is a trademark, and ANTI-FLAG and FLAG are registered trademarks trademarks of Sigma-Aldrich® Biotechnology LP and Sigma-Aldrich Co.

**Academic and Non-Profit Laboratory Assurance Letter**

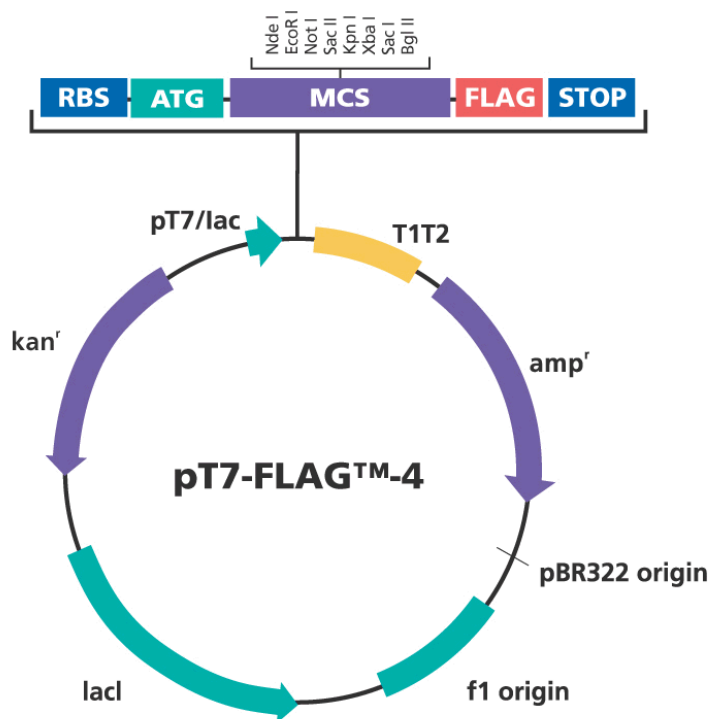
The T7 system is based on technology developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy and is the subject of U.S. Patent No. 5,693,489 (expiration date, December 2, 2014) assigned to Brookhaven Science Associates, LLC. (BSA). BSA will grant a nonexclusive license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

**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).

## pT7-FLAG-4 (6.4 kb)



### Multiple Cloning Site (pT7-FLAG-4)

Nde I Met EcoR I Not I Sac II Kpn I Xba I Sac I Bgl II  
 CAT ATG AAG CTT CTC GAG AAT TCG CGG CCG CGG TAC CTC TAG AGC TCA GAT CTG TC  
 GTA TAC TTC GAA GAG CTC TTA AGC GCC GGC GCC ATG GAG ATC TCG AGT CTA GAC AG

**FLAG Peptide Sequence**

Asp Tyr Lys Asp Asp Asp Asp Lys STOP  
 GAC TAC AAG GAC GAT GAC GAC AAG TGA  
 CTG ATG TTC CTG CTA CTG CTG TTC ACT

AH,PHC 09/10-1

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

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.