

Product Information

pT7-FLAG-4 Expression Vector

Catalog Number **P 9743**
 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^{1,2} 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) cells, Catalog Numbers B2935, B3310, or B3435. 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^r* and *kan^r* 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 A 2220. Please visit www.sigma-aldrich.com for a complete listing of resins and affinity capture plates.

Reagents

- pT7-FLAG-4 Expression Vector, 10 µg, 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 µg, 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 $-20\text{ }^{\circ}\text{C}$.

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.

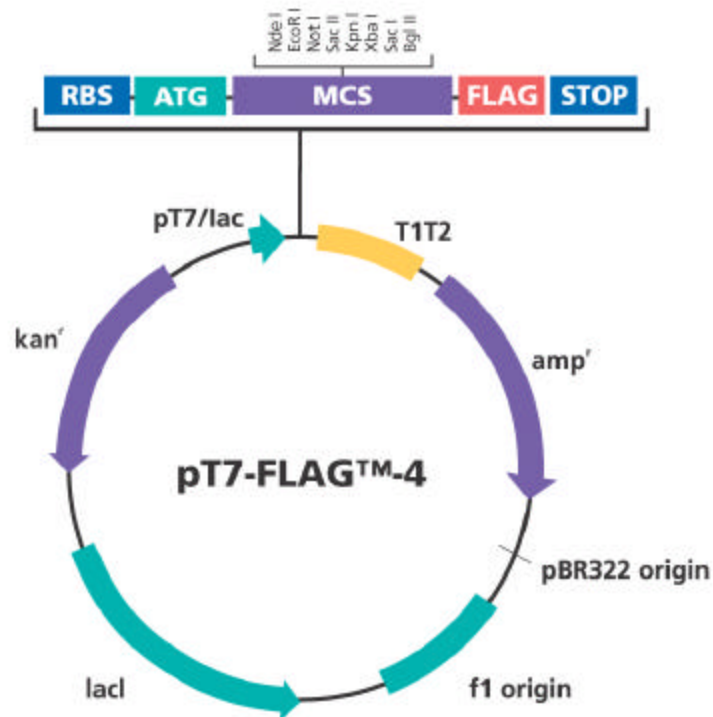
pT7-FLAG-4

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
β-lactamase (<i>amp^r</i>)	823-1680
pBR322 ori	1888-2007
f1 ori	2671-3134
<i>lacI</i>	3812-4894
APH-II (<i>kan^r</i>)	5639-6451

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 CCG CCG CCG TAC CTC TAG AGC TCA GAT CTG TC
 GTA TAC TTC GAA GAG CTC TTA ABC GCC GGC GGC 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

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 patent applications assigned to Brookhaven Science Associates, LLC. (BSA). BSA will grant a non-exclusive 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 Economic Development and Technology Transfer, Brookhaven National Laboratory, Bldg. 475D, P.O. Box 5000, Upton, New York 11973-5000, telephone (516)-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 you may make of them.
3. You may refuse this license by returning the enclosed materials unused. By keeping our using the enclosed materials, you agree to be bound to the terms of this license

LICENSE AGREEMENT

The enclosed DNA expression vector and/or antibody are specifically adapted for a method of producing selected protein molecules covered by one or more of the following patents owned by Sigma-Aldrich Co.: U.S. Patent Nos. 5,011,912, 4,703,004, 4,782,137 and 4,851,341; EP Patent No. 150,126 (Austria, Belgium, Switzerland, France, United Kingdom, Italy, Netherlands and Sweden); EP Patent No. 335,899 (Belgium, Switzerland, Germany, France, United Kingdom, Italy, Luxembourg and Sweden); German Patent No. P3584260.1; Canadian Patent No. 1,307,752; and Japanese Patent Nos. 1,983,150 and 2,665,359. Your payment includes a limited license under these patents to make only the following uses of these products:

A. Vector License: You may use the enclosed vector to transform cells to produce proteins containing the amino acid sequence DYKDDDDK for research purposes provided, however, such research purposes do not include binding an unlicensed antibody to any portion of this amino acid sequence nor using such proteins for the preparation of antibodies having an affinity for any portion of this amino acid sequence.

B. Antibody License: You may only use the enclosed antibody for research purposes to perform a method of producing a protein in which the protein is expressed in a host cell and purified by use of the antibody in accordance with a claim in one of the above patents in force in a country where the use actually occurs so long as: (1) you perform such method with a DNA expression vector licensed from Sigma-Aldrich Co.; and (2) you do not bind (or allow others to bind) an unlicensed antibody to any DYKDDDDK epitope of any fusion protein that is produced by use of the method.

This license does not include any rights under any other patents. You are not licensed to use the vector and/or antibody in any manner or for any purpose not recited above. As used above, the term "unlicensed antibody" means any antibody which Sigma-Aldrich Co. has not expressly licensed pursuant to Paragraph B, above. Sigma-Aldrich Co. hereby expressly retains all rights in the above listed patents not expressly licensed hereunder.

If the terms and conditions of this License Agreement are acceptable to you, then you may open the vessel(s) containing the vector and/or antibody and, through such act of opening a vessel, will have shown your acceptance to these terms and conditions.

If the terms and conditions of this License Agreement are not acceptable to you, then please return the vessel(s) unopened to Sigma-Aldrich Co. for a complete refund of your payment.

For additional licensing information or to receive a copy of any of the above patents, please contact the Sigma-Aldrich Co. licensing department at telephone number 314-771-5765.

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

ND,PHC 11/05-1

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.