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™-SBP-1 Expression Vector

Catalog Number **P3871**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The pT7-FLAG-SBP-1 Expression Vector is a 4931 bp *Escherichia coli* expression vector used for cloning and cytoplasmic expression of a properly inserted open reading frame. The protein is expressed as an N-terminal Met-FLAG®, C-terminal SBP fusion protein containing the FLAG epitope (DYKDDDDK)[1] and the 38 amino acid Streptavidin Binding Peptide (SBP) tag.[2] This vector requires the use of *E. coli* cells containing the DE3 lysogen as a source of the T7 polymerase,[3] such as BL21(DE3)T1^{R} cells, Catalog Number B2935.

pT7-FLAG-SBP-1 may be used in conjunction with the Director™ Universal PCR System, Catalog Number RDC1, for a simple, rapid and universal method to directionally clone PCR products. The MCS has been optimized for use of the well-validated *Hin*d III/*Bgl* II pair of 5' overhang producing restriction enzymes often used in the Director Universal PCR System. The N-terminal FLAG fusion protein may be detected using the ANTI-FLAG® M2 monoclonal antibody, Catalog Number F3165, and purified using the ANTI-FLAG M2 Affinity Gel, Catalog Number A2220. The SBP tag may be detected using Streptavidin-Alkaline Phosphatase, Catalog Number S2890, or Streptavidin-Peroxidase, Catalog Number S5512. The fusion protein may also be purified using Streptavidin-Agarose, Catalog Number S1638. Sigma offers a wide selection of ANTI-FLAG and streptavidin products; please visit www.sigma-aldrich.com for a complete listing of antibody conjugates, resins, and affinity capture plates.

The following table provides map positions to key features in the pT7-FLAG-SBP-1 vector. Sequence verification of the MCS and C-terminal junction 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.

pT7- FLAG-SBP-1 Features

Feature	Map Position
pT7 Promoter	79-98
lacO	99-118
Recommended 5' primer sequence binding site	31-53
Ribosomal Binding Site	150-155
FLAG epitope	165-188
SBP tag	218-337
MCS	186-217
C-24 Sequencing Primer Binding Site	360-383
T1/T2 terminator	391-761
β-lactamase (amp^{r})	860-1717
pBR322 ori	1925-2044
f1 ori	2708-3171
lacI	3849-4931

Reagents

- pT7-FLAG-SBP-1 Expression Vector
  Catalog Number E1030
  10 µg, 0.5 mg/ml in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.
- pT7-SBP-1+ BAP Control Vector
  Catalog Number C0364
  1 µg, 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 and storage at –20 °C is recommended.

References

1. Hopp, T. V., et al., *Bio/Technology*, **6**, 1204-1210 (1988).
2. Keefe, A. D., et al., *Protein Expr. Purif.*, **23,** 440-446 (2001).
3. Studier, F. W., et al., *Methods Enzymol*. **185**, 60-89 (1990).

Academic and Non-Profit Laboratory Assurance Letter

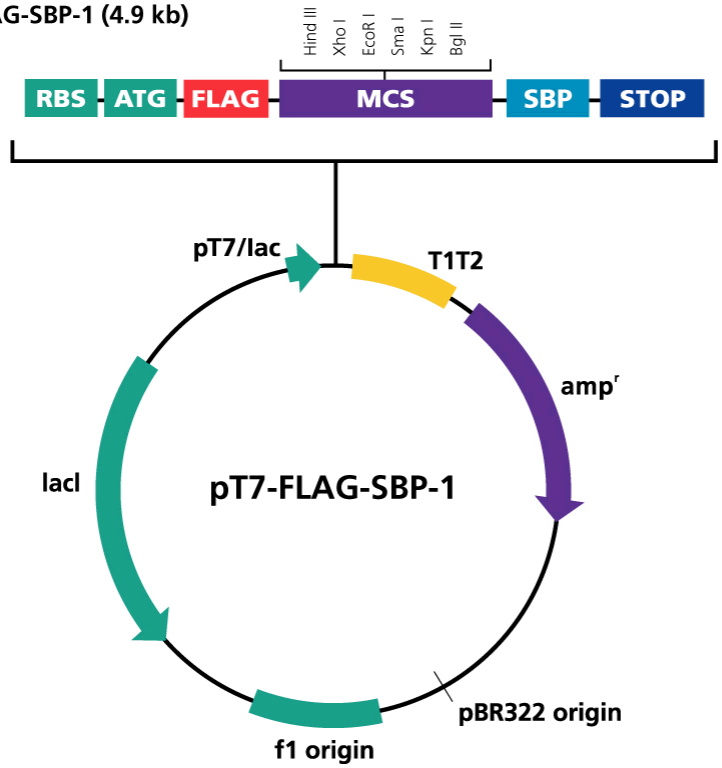
The T7 system is based on technology developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy and is the subject of U.S. Patent No. 5,693,489 (expiration date, December 2, 2014) assigned to Brookhaven Science Associates, LLC. (BSA). BSA will grant a nonexclusive license 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-SBP-1 (4.9 kb)



Multiple Cloning Site

(pT7-FLAG-SBP-1)

FLAG Peptide Sequence

Met	Asp	Tyr	Lys	Asp	Asp	Asp	Asp	Lys

Xho I EcoR I Sma I Kpn I Bgl II

```
ATG GAC TAC AAG GAC GAT GAC GAC AAG CTT CTC GAG AAT TCC CGG GTA CCA GAT CT*
TAC CTG ATG TTC CTG CTA CTG CTG TTC GAA GAG CTC TTA AGG GCC CAT GGT CTA GA
                                Hind III
```

SBP Sequence

Met	Asp	Glu	Lys	Thr	Thr	Gly	Trp	Arg	Gly	Gly	His	Val	Val	Glu	Gly	Leu	Ala	Gly

```
ATG GAC GAA AAA ACC ACC GGT TGG CGT GGT GGT CAC GTT GTT GAA GGT CTG GCT GGT
TAC CTG CTT TTT TGG TGG CCA ACC GCA CCA CCA GTG CAA CAA CTT CCA GAC CGA CCA
```

SBP Sequence

Glu	Leu	Glu	Gln	Leu	Arg	Ala	Arg	Leu	Glu	His	His	Pro	Gln	Gly	Gln	Arg	Glu	Pro	STOP

```
GAA CTG GAA CAG CTG CGT GCT CGT CTG GAA CAC CAC CCG CAG GGT CAG CGT GAA CCG TAA
CTT GAC CTT GTC GAC GCA CGA GCA GAC CTT GTG GTG GGC GTC CCA GTC GCA CTT GGC ATT
```

*For presentation purposes, the reading frame changes here. Please note that cloning into the *Hind* III and *Bgl* II sites produces an in-frame dual tagged fusion protein.