

pTWIN1



1-800-632-7799
info@neb.com
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N6951S 002141016101

N6951S

10 µg Lot: 0021410 Exp: 10/16
200 µg/ml Store at -20°C

Description: pTWIN1 is an *E. coli* expression vector which can be used with the IMPACT™ Kit (NEB #E6901). pTWIN vectors are designed for protein purification or for the isolation of proteins with an N-terminal cysteine and/or a C-terminal thioester (1). A polylinker in the vector is designed for the in-frame fusion of a target gene between the modified Ssp DnaB (2) and Mxe GyrA inteins (3). The presence of the chitin binding domain from *Bacillus circulans* (4,5) facilitates purification. The double-stranded vector is 7,375 base pairs in length.

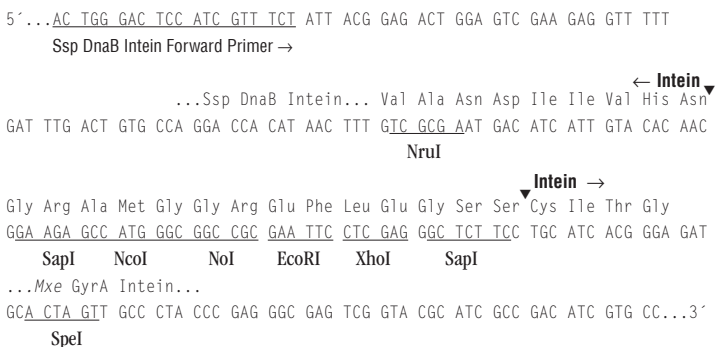
Source: pTWIN1 contains two mini-inteins, one derived from the *Synechocystis sp* DnaB intein (154 amino acids) (6) and the other from the *Mycobacterium xenopi* GyrA intein (198 amino acids) (7).

Supplied in: 10 mM Tris-HCl (pH 8.0), 1 mM EDTA.

Features of pTWIN1:

- A pBR322 derivative
- The SapI sites should be used for directional cloning of both the 5' and 3' ends of an insert.

Polylinker Region: pTWIN1



- Expression of the fusion gene is under the control of the T7 promotor (8) and is regulated by IPTG due to the presence of a *lacI* gene.
- Expression requires an *E. coli* host that carries the T7 RNA Polymerase gene [e.g., T7 Express Competent *E. coli* (High Efficiency), (NEB #C2566) or BL21(DE3) Competent *E. coli*, (NEB #C2527) and derivatives].
- Origin of DNA replication from the bacteriophage M13 allows for the production of single-stranded DNA by helper phage superinfection of cells bearing the plasmid.

- Thiol-induced cleavage of the Mxe GyrA intein is dependent on the amino acids adjacent to the intein. The amino acid residues M or Y at the C-terminus of the target protein is recommended for use with this intein.
- Controllable cleavage of the Ssp DnaB intein is dependent on the amino acids adjacent to the intein. The amino acid residues CRA or GRA at the N-terminus of the target protein is recommended for use with this intein.
- Ampicillin resistance.

Recommended Buffers

- Cell Lysis Buffer: 50 mM Tris-HCl (pH 8.5) containing 500 mM NaCl.
- Ssp DnaB Intein Cleavage Buffer: 50 mM Tris-HCl (pH 6.0) containing 500 mM NaCl.
- Mxe GyrA Intein Cleavage Buffer: 50 mM Tris-HCl (pH 8.5) containing 500 mM NaCl and 50 mM 2-mercaptoethanesulfonic acid.

(see other side)

CERTIFICATE OF ANALYSIS

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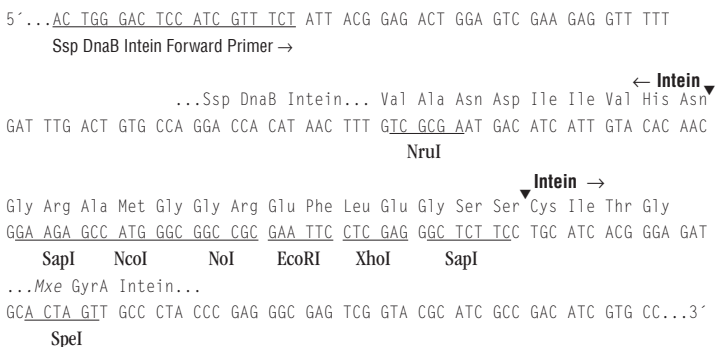
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References:

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Additional information such as vector sequences and frequently asked questions, are available at www.neb.com.



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