pTWIN1

1-800-632-7799
info@neb.com
www.neb.com

N6951S 002141116111

N6951S

10 µg **Lot: 0021411** **Exp: 11/16**

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.
- 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 *lac*I 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.

Polylinker Region: pTWIN1

```
5´...AC TGG GAC TCC ATC GTT TCT ATT ACG GAG ACT GGA GTC GAA GAG GTT TTT
      Ssp DnaB Intein Forward Primer →
                                                          ← Intein
                    ...Ssp DnaB Intein... Val Ala Asn Asp Ile Ile Val His Asn
GAT TTG ACT GTG CCA GGA CCA CAT AAC TTT GTC GCG AAT GAC ATC ATT GTA CAC AAC
                                          NruI
                                                    Intein →
Gly Arg Ala Met Gly Gly Arg Glu Phe Leu Glu Gly Ser Ser Cys Ile Thr Gly
GGA AGA GCC ATG GGC GGC CGC GAA TTC CTC GAG GGC TCT TCC TGC ATC ACG GGA GAT
    SapI     NcoI        NoI     EcoRI    XhoI      SapI
...Mxe GyrA Intein...
GCA CTA GTT GCC CTA CCC GAG GGC GAG TCG GTA CGC ATC GCC GAC ATC GTG CC...3´
     SpeI
```

- 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

pTWIN1

1-800-632-7799
info@neb.com
www.neb.com

N6951S 002141116111

N6951S

10 µg Lot: 0021411 Exp: 11/16

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.
- 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 *lac*I 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.

Polylinker Region: pTWIN1

5´...AC TGG GAC TCC ATC GTT TCT ATT ACG GAG ACT GGA GTC GAA GAG GTT TTT
Ssp DnaB Intein Forward Primer →
← Intein
...Ssp DnaB Intein... Val Ala Asn Asp Ile Ile Val His Asn
GAT TTG ACT GTG CCA GGA CCA CAT AAC TTT GTC GCG AAT GAC ATC ATT GTA CAC AAC
NruI
Intein →
Gly Arg Ala Met Gly Gly Arg Glu Phe Leu Glu Gly Ser Ser Cys Ile Thr Gly
GGA AGA GCC ATG GGC GGC CGC GAA TTC CTC GAG GGC TCT TCC TGC ATC ACG GGA GAT
SapI NcoI NoI EcoRI XhoI SapI
...Mxe GyrA Intein...
GCA CTA GTT GCC CTA CCC GAG GGC GAG TCG GTA CGC ATC GCC GAC ATC GTG CC...3´
SpeI

- 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

References:

1. Evans, T.C., Benner, J., and Xu, M.-Q. (1999) The cyclization and polymerization of bacterially expressed proteins using modified self-splicing inteins. *J. Biol. Chem.* 274, 18359–18363.
2. Mathys, S., Evans, T.C., Chute, I.C., Wu, H., Chong, S., Benner, J., Liu, X.-Q. and Xu, M.-Q. (1999). Characterization of a self-splicing mini-intein and its conversion into autocatalytic N-and C-terminal cleavage elements: facile production of protein building blocks for protein ligation. *Gene* 231, 1–13.
3. Evans, T.C., Benner, J. and Xu, M.-Q. (1998) Semisynthesis of cytotoxic proteins using a modified protein splicing element. *Protein Sci.* 7, 2256–2264.
4. Chong, S., Mersha, F.B., Comb, D.G., Scott, M. E., Landry, D., Vence, L.M., Perler, F.B., Benner, J., Kucera, R.B., Hirvonen, C.A., Pelletier, J.J., Paulus, H. and Xu, M.-Q. (1997) Single-column purification of free recombinant proteins using a self-cleavable affinity tag derived from a protein splicing element. *Gene* 192, 271–281.
5. Watanabe, T., Ito, Y.,Yamada, T., Hashimoto, M., Sekine, S. and Tanaka, H. (1994). The role of the C-terminal domain and type III domains of chitinase A1 from *Bacillus circulans* WL-12 in chitin degradation. *J. Bacteriol.* 176, 4465–4472.
6. Wu, H., Xu, M.-Q. and Liu, X.-Q. (1998) Protein trans-splicing and functional mini-inteins of a cyanobacterial DnaB intein. *Biochem. Biophys, Acta* 1387, 422–432.
7. Telenti, A., Southworth, M., Alcaide, F., Daugelat, S., Jacobs, W.R. Jr. and Perler, F.B. (1997) The *Mycobacterium xenopi* GyrA protein splicing element: Characterization of a minimal intein. *J. Bacteriol.* 179, 6378–6382.
8. Dubendorff, J.W. and Studier, F.W. (1991) Controlling basal expression in an inducible T7 expression system by blocking the target T7 promoter with *lac* repressor. *J. Mol. Biol.* 219, 45–59.

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