pTWIN2

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

N6952S 001111114111

N6952S

10 µg **Lot: 0011111** **Exp: 11/14**

200 mg/ml **Store at –20°C**

Description: pTWIN2 is an *E. coli* expression vector which can be used with the IMPACT™ Kit (NEB #E6901) (1). A polylinker in the vector is designed for the in-frame fusion of a target gene between the modified *Ssp* DnaB (2) and *Mth* RIR1 inteins (3). The presence of the chitin binding domain from *Bacillus circulans* (4,5) facilitates purification. 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). The double-stranded vector is 7192 base pairs in length.

Source: pTWIN2 contains two mini-inteins, one derived from the *Synechocystis sp* DnaB intein (154 amino acids) (6) and the other from the *Methanobacterium thermoautotrophicum* intein (134 amino acids) (7).

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

Features of pTWIN2:

- A pBR322 derivative
- The SapI sites are recommended 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.
- Thiol-induced cleavage of the Mth RIR1 intein is dependent on the amino acids adjacent to the intein. The amino acid Gly 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 acids CRA or GRA at the N-terminus of the target protein is recommended for use with this intein.
- Ampicillin resistance.

Polylinker Region: pTWIN2

```
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 Val Ser Gly Asp Thr
GGA AGA GCC ATG GGC GGC CGC GAA TTC CTC GAG GGC TCT TCC TGC GTA TCC GGT GAC ACC ATT
      SapI  NcoI       NotI     EcoRI   XhoI      SapI
...Mth RIR1 Intein...
GTA ATG ACT AGT GGC GGT CCG CGC ACT GTG GCT GAA CTG GAG GGC AAA CCG TTC ACC...3´
        SpeI                                  ← Mth RIR1 Intein Reverse Primer
```

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 7.0) containing 500 mM NaCl.
- Mth RIR1 Intein Cleavage Buffer: 50 mM Tris-HCl (pH 8.5) containing 500 mM NaCl and 50 mM 2-mercaptoethanesulfonic acid.

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.

(see other side)

CERTIFICATE OF ANALYSIS

pTWIN2

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

N6952S 001111114111

N6952S

10 µg Lot: 0011111 Exp: 11/14

200 mg/ml Store at –20°C

Description: pTWIN2 is an *E. coli* expression vector which can be used with the IMPACT™ Kit (NEB #E6901) (1). A polylinker in the vector is designed for the in-frame fusion of a target gene between the modified *Ssp* DnaB (2) and *Mth* RIR1 inteins (3). The presence of the chitin binding domain from *Bacillus circulans* (4,5) facilitates purification. 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). The double-stranded vector is 7192 base pairs in length.

Source: pTWIN2 contains two mini-inteins, one derived from the *Synechocystis sp* DnaB intein (154 amino acids) (6) and the other from the *Methanobacterium thermoautotrophicum* intein (134 amino acids) (7).

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

Features of pTWIN2:

- A pBR322 derivative
- The SapI sites are recommended 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.
- Thiol-induced cleavage of the Mth RIR1 intein is dependent on the amino acids adjacent to the intein. The amino acid Gly 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 acids CRA or GRA at the N-terminus of the target protein is recommended for use with this intein.
- Ampicillin resistance.

Polylinker Region: pTWIN2

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 Val Ser Gly Asp Thr
GGA AGA GCC ATG GGC GGC CGC GAA TTC CTC GAG GGC TCT TCC TGC GTA TCC GGT GAC ACC ATT
SapI NcoI NotI EcoRI XhoI SapI
...Mth RIR1 Intein...
GTA ATG ACT AGT GGC GGT CCG CGC ACT GTG GCT GAA CTG GAG GGC AAA CCG TTC ACC...3´
SpeI ← Mth RIR1 Intein Reverse Primer

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 7.0) containing 500 mM NaCl.
- Mth RIR1 Intein Cleavage Buffer: 50 mM Tris-HCl (pH 8.5) containing 500 mM NaCl and 50 mM 2-mercaptoethanesulfonic acid.

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.

(see other side)

CERTIFICATE OF ANALYSIS

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. (1999) The *in vitro* ligation of bacterially expressed proteins using an intein from *Methano-bacterium thermo-autotrophicum. J. Biol. Chem.* 274, 3923–3926.
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. Smith, D.R., et al. (1997) Complete genome sequence of *Methanobacterium thermo-autotrophicum* ΔH: functional analysis and comparative genomics. *J. Bacteriol.* 179, 7135–7155.
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.

RESEARCH USE ASSURANCE STATEMENT

The buyer and user have a non-exclusive sub-license to use this system or any component thereof for RESEARCH PURPOSES ONLY, based upon agreement to the following assurances:

Transfer of the host cells that contain the cloned copy of the T7 gene 1 to third parties is explicitly prohibited. This information applies to *E. coli* ER2566, ER2833, ER3011, ER3012, ER3013 and ER3021, SHuffle T7, SHuffle T7 *LysY*, SHuffle T7 Express, SHuffle T7 Express *LysY* and their competent derivatives, C2566, C2833, C3009, C3010, C3013, C3016, 3021, C3022, C3026, C3027, C3029 and C3030 when povided separately or when provided in combination with appropriate vectors for said systems.

A license to use this system or any components thereof for commercial purposes may be obtained from New England Biolabs, Inc.

Commercial Laboratory Buyer and User:
Use of the host cells ER2566, ER2833, C2566, C2833, C3009, C3010, C3013, C3016, C3021, C3022, C3026, C3027, C3029, C3030, SHuffle T7, SHuffle T7 *LysY*, SHuffle T7 Express, SHuffle T7 Express *LysY* or their competent derivatives that contain the cloned copy of T7 gene 1, the gene for T7 RNA polymerase for any purpose other than in combination with either a T7/MAL or T7/IMPACT vector is explicitly prohibited.

Use of the host cells that may contain the cloned copy of the T7 gene 1, the gene for T7 RNA polymerase with any other vector(s) containing a T7 promoter to direct the production of RNA or protein requires a license from Brookhaven National Laboratory. Information about research-use or commercial-use license agreement may be obtained from the Office of Intellectual Property and Sponsored Research, Brookhaven Natonal Laboratory, Buildng 475D, P.O. Box 5000, Upton, New York 11973-5000. Tel.: 631-344-7134. Fax: 631-344-3729.

You may refuse this non-exclusive research license agreement by returning the enclosed materials unused. By keeping or using the enclosed materials, you agree to be bound by the terms of this sub-license.

ACADEMIC AND NON-PROFIT LABORATORY ASSURANCE LETTER

The T7 expression system is based on technology developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy and is the subect of patent applications assigned to Brookhaven Science Associates, LLC (BSA). BSA will grant a non-exclusive license for use of this technology, including the enclosed materials, based upon the following assurances:

1. These materials are to be used for non-commercial research purposes only. A separate license is required for any commercial use, including the 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 Intellectual Property and Sponsored Research, Brookhaven National Laboratory, Building 475D, P.O. Box 5000, Upton, New York 11973-5000. Tel: 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 recipient receives a copy of this license and agrees to be bound by the terms. This limitation applies to strains ER2566, ER2833, C2566, C2833, C3009, C3010, C3013, C3016, C3021, C3022, C3026, C3027, C3029, C3030, BL21(DE3), BL21(DE)pLysS and BL21(DE3)pLysE, SHuffle T7, SHuffle T7 *LysY*, SHuffle T7 Express, SHuffle T7 Express *LysY* and ther competent derivatives and any derivatives you may make of them, including such strains containing recombinant vectors.

You may refuse this license by returning the enclosed materials unused. By keeping or using the enclosed materials, you agree to be bound by the terms of this license.

U.S. Patent Nos. 5,496,714, 5,834,247

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. (1999) The *in vitro* ligation of bacterially expressed proteins using an intein from *Methano-bacterium thermo-autotrophicum. J. Biol. Chem.* 274, 3923–3926.
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. Smith, D.R., et al. (1997) Complete genome sequence of *Methanobacterium thermo-autotrophicum* ΔH: functional analysis and comparative genomics. *J. Bacteriol.* 179, 7135–7155.
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.

This product is sold for research use only and not for resale in any form. Commercial use of this product may require a license. For license information, please contact the Licensing Office, New England Biolabs, Inc., 240 County Road, Ipswich, MA 01938.

RESEARCH USE ASSURANCE STATEMENT

The buyer and user have a non-exclusive sub-license to use this system or any component thereof for RESEARCH PURPOSES ONLY, based upon agreement to the following assurances:

Transfer of the host cells that contain the cloned copy of the T7 gene 1 to third parties is explicitly prohibited. This information applies to *E. coli* ER2566, ER2833, ER3011, ER3012, ER3013 and ER3021, SHuffle T7, SHuffle T7 *LysY*, SHuffle T7 Express, SHuffle T7 Express *LysY* and their competent derivatives, C2566, C2833, C3009, C3010, C3013, C3016, 3021, C3022, C3026, C3027, C3029 and C3030 when povided separately or when provided in combination with appropriate vectors for said systems.

A license to use this system or any components thereof for commercial purposes may be obtained from New England Biolabs, Inc.

Commercial Laboratory Buyer and User:
Use of the host cells ER2566, ER2833, C2566, C2833, C3009, C3010, C3013, C3016, C3021, C3022, C3026, C3027, C3029, C3030, SHuffle T7, SHuffle T7 *LysY*, SHuffle T7 Express, SHuffle T7 Express *LysY* or their competent derivatives that contain the cloned copy of T7 gene 1, the gene for T7 RNA polymerase for any purpose other than in combination with either a T7/MAL or T7/IMPACT vector is explicitly prohibited.

Use of the host cells that may contain the cloned copy of the T7 gene 1, the gene for T7 RNA polymerase with any other vector(s) containing a T7 promoter to direct the production of RNA or protein requires a license from Brookhaven National Laboratory. Information about research-use or commercial-use license agreement may be obtained from the Office of Intellectual Property and Sponsored Research, Brookhaven Natonal Laboratory, Buildng 475D, P.O. Box 5000, Upton, New York 11973-5000. Tel.: 631-344-7134. Fax: 631-344-3729.

You may refuse this non-exclusive research license agreement by returning the enclosed materials unused. By keeping or using the enclosed materials, you agree to be bound by the terms of this sub-license.

ACADEMIC AND NON-PROFIT LABORATORY ASSURANCE LETTER

The T7 expression system is based on technology developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy and is the subect of patent applications assigned to Brookhaven Science Associates, LLC (BSA). BSA will grant a non-exclusive license for use of this technology, including the enclosed materials, based upon the following assurances:

1. These materials are to be used for non-commercial research purposes only. A separate license is required for any commercial use, including the 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 Intellectual Property and Sponsored Research, Brookhaven National Laboratory, Building 475D, P.O. Box 5000, Upton, New York 11973-5000. Tel: 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 recipient receives a copy of this license and agrees to be bound by the terms. This limitation applies to strains ER2566, ER2833, C2566, C2833, C3009, C3010, C3013, C3016, C3021, C3022, C3026, C3027, C3029, C3030, BL21(DE3), BL21(DE)pLysS and BL21(DE3)pLysE, SHuffle T7, SHuffle T7 *LysY*, SHuffle T7 Express, SHuffle T7 Express *LysY* and ther competent derivatives and any derivatives you may make of them, including such strains containing recombinant vectors.

You may refuse this license by returning the enclosed materials unused. By keeping or using the enclosed materials, you agree to be bound by the terms of this license.

U.S. Patent Nos. 5,496,714, 5,834,247

Page 2 (N6952S)