pTXB1 Vector

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

N6707S 005130916091

N6707S

10 µg **Lot: 0051309** **Exp: 9/16**

0.20 mg/ml **Store at –20°C**

Description: pTXB1 is an *E. coli* expression vector in the IMPACT™ Kit (1,2). It is designed for the in-frame insertion of a target gene into the polylinker upstream of the Mxe intein/chitin binding domain (27 kDa)(2,3). The fusion protein is bound to chitin beads and the thiol-induced cleavage activity of the intein releases the target protein. pTXB vectors are recommended for use in intein-mediated protein ligation and C-terminal labeling (2). This double stranded vector is 6,706 base pairs in length.

Source: pTXB1 contains the intein (198 amino acids) from the *Mycobacterium xenopi* GyrA gene (2,4).

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

Features of this vector:

- The NdeI site in the polylinker contains an ATG sequence for translation initiation.
- The SapI site should be used for cloning of the 3´ end of the insert. Use of the SapI site allows cloning of the target protein adjacent to the intein, resulting in cleavage of the target protein without any additional amino acids at its C-terminus. (See NEB's web site for primer design).
- Expression of the fusion gene is under the control of an IPTG-inducible T7 promoter (5).
- A pBR322 derivative with a ColE1replication origin.
- Origin of DNA replication from bacteriophage M13, which allows for the production of single-stranded DNA by helper phage superinfection of cells bearing the plasmid (M13K07 Helper Phage, NEB #N0315).
- Ampicillin resistance
- Other IMPACT vectors are available which allow for fusion of a target gene to N- or C-terminus of an intein. The cleavage reaction may be induced by thiol reagent or temperature/pH shift.
- Companion vector pTXB3 (NEB #N6708) contains an NcoI site in place of NdeI.
- A wide range of *E. coli* host strains: T7 Express Competent *E. coli* (High Efficiency) (NEB #C2566) or BL21(DE3) Competent *E. coli* (NEB #C2527) and derivatives.

Polylinker Region:

pTXB1

T7 Universal Primer →

```
5´...CGG GGA TCT CGA TCC CGC GAA ATT AAT ACG ACT CAC TAT AGG GGA ATT GTG AGC
                                                T7 Promoter          lac operator

GGA TAA CAA TTC CCC TCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA GAT ATA
                    XbaI                                 ShineDalgarno

                                                                  Intein →
    Met Ala Ser Ser Arg Val Asp Gly Gly Arg Glu Phe Leu Glu Gly Ser Ser▼Cys1
CAT ATG GCT AGC TCG CGA GTC GAC GGC GGC CGC GAA TTC CTC GAG GGC TCT TCC TGC
 NdeI    NheI    NruI    SalI      NotI      EcoRI    XhoI      SapI

ATC ACG GGA GAT GCA CTA GTT GCC CTA CCC GAG GGC GAG TCG GTA
                    SpeI

CGC ATC GCC GAC ATC GTG CCG ...3´
```

(see other side)

CERTIFICATE OF ANALYSIS

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                                                T7 Promoter          lac operator

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                    XbaI                                 ShineDalgarno

                                                                  Intein →
    Met Ala Ser Ser Arg Val Asp Gly Gly Arg Glu Phe Leu Glu Gly Ser Ser▼Cys1
CAT ATG GCT AGC TCG CGA GTC GAC GGC GGC CGC GAA TTC CTC GAG GGC TCT TCC TGC
 NdeI    NheI    NruI    SalI      NotI      EcoRI    XhoI      SapI

ATC ACG GGA GAT GCA CTA GTT GCC CTA CCC GAG GGC GAG TCG GTA
                    SpeI

CGC ATC GCC GAC ATC GTG CCG ...3´
```

(see other side)

CERTIFICATE OF ANALYSIS

References:

1. 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.
2. 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.
3. Watanabe, T., Ito, Y., Yamada, T., Hasmimoto, 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.
4. 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.
5. 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.

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

References:

1. 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.
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