pTYB2 Vector

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

N6702S 006110314030

N6702S

10 µg **Lot: 0061103** **Exp: 3/14**

200 µg/ml **Store at –20°C**

Description: pTYB2 is an *E. coli* cloning and expression vector (7474 bp) used in the IMPACT™ Kit which allows the overexpression of a target protein as a fusion to a self-cleavable affinity tag (1,2). This C-terminal fusion vector is designed for the in-frame insertion of a target gene into the polylinker upstream of an intein tag (the *Sce* VMA intein/chitin binding domain, 55 kDa) (1,2) This results in the fusion of the C-terminus of the target protein to the N-terminus of the intein tag. Thiol-induced self-cleavage of the intein releases the target protein from the chitin-bound intein tag, resulting in a single column purification of the target protein.

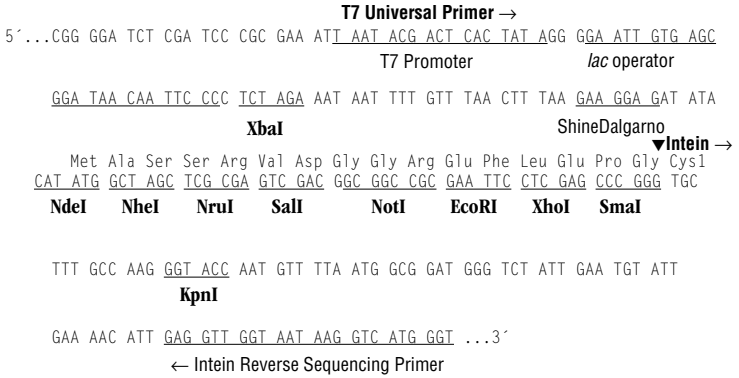
For fusion of the N-terminus of the target protein to the intein tag, use pTYB11 (NEB #N6901) or pTYB12 (NEB #N6902). This vector can be used in conjuction with pTYB12 (NEB #N6902) to test which fusion construction (N-terminal or C-terminal) maximizes the expression and yield of a target protein (3).

Source: pTYB2 is isolated from an *E. coli* strain (r- m-) by a standard plasmid purification procedure.

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

Features of pTYB2:

Multiple Cloning Sites (MCS):



- The NdeI site in the polylinker contains an ATG sequence for translation initiation.
- The SmaI site is used for cloning the 3´ end of the target gene and will yield a target protein with a single glycine residue added to its C-terminus after cleavage of the intein.
- Unique sites are indicated in bold.
- A pBR322 derivative with a ColE1 replication origin.
- Expression of the fusion gene is under the control of an IPTG-inducible T7 promoter (4).
- 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].
- Ampicillin resistance.
- Origin of DNA replication from the bacterio-phage M13 allows for the production of single-stranded DNA by helper phage superinfection of cells bearing the plasmid. M13K07 Helper Phage is available from (NEB #N0315).
- 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.
- The sites in the polylinker region are identical to or compatible with (i.e., NheI of pTYB2 and SpeI of pTYB12) those of pTYB12 (NEB #N6902). This allows the same amplified target gene to be cloned into either vector for optimizing protein expression. Vector derived residues may be present at the N- and/or C-termini of the target proteins.
- Companion vectors (pTYB1, pTYB3, pTYB4) differ only in the sites present in the polylinker.

(see other side)

CERTIFICATE OF ANALYSIS

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

Features of pTYB2:

Multiple Cloning Sites (MCS):

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 Pro Gly Cys1
CAT ATG GCT AGC TCG CGA GTC GAC GGC GGC CGC GAA TTC CTC GAG CCC GGG TGC
NdeI NheI NruI SalI NotI EcoRI XhoI SmaI
TTT GCC AAG GGT ACC AAT GTT TTA ATG GCG GAT GGG TCT ATT GAA TGT ATT
KpnI
GAA AAC ATT GAG GTT GGT AAT AAG GTC ATG GGT ...3´
← Intein Reverse Sequencing Primer

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- The SmaI site is used for cloning the 3´ end of the target gene and will yield a target protein with a single glycine residue added to its C-terminus after cleavage of the intein.
- Unique sites are indicated in bold.
- A pBR322 derivative with a ColE1 replication origin.
- Expression of the fusion gene is under the control of an IPTG-inducible T7 promoter (4).
- 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].
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(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, 277–281.
2. 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.
3. Chong, S., Montello, G.E., Zhang, A., Cantor, E.J., Liao, W., Xu, M -Q, Benner, J. (1998) Utilizing the C-terminal cleavage activity of a protein splicing element to purify recombinant proteins in a single chromatographic step. *Nucl. Acids Res.* 26, 5109–5115.
4. 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, 277–281.
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