pTYB3 Vector

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

N6703S 003111113111

N6703S

10 µg **Lot: 0031111** **Exp: 11/13**

200 µg/ml **Store at –20°C**

Description: pTYB3 is an *E. coli* expression vector (7,477 bp) used in the IMPACT™ Kit (1). This C-terminal fusion vector is designed for the in-frame insertion of a target gene into a 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 (3).

For the fusion of the N-terminus of the target protein to the intein tag, use pTYB11 (NEB #N6901) or pTYB12 (NEB #N6902).

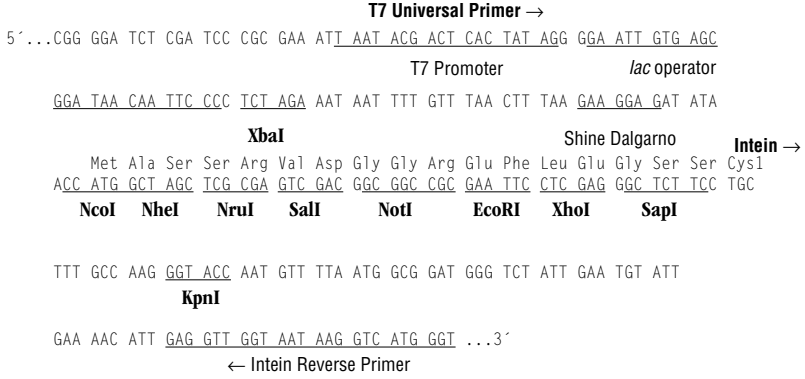
Source: pTYB3 is isolated from an *E. coli* strain (r- m-) by a standard plasmid purification procedure.

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

Features of pTYB3:

- The NcoI site in the polylinker contains an ATG sequence for translation initiation.
- 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.
- Unique sites are indicated in **bold**.
- Expression of the fusion gene is under the control of an IPTG-inducible T7 promoter (4).

Polylinker Region:



- 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)
- A pBR322 derivative with a ColE1 replication origin.
- 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 vectors (pTYB1, pTYB2, pTYB4) differ only in the sites present in the polylinker.
- 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.

(see other side)

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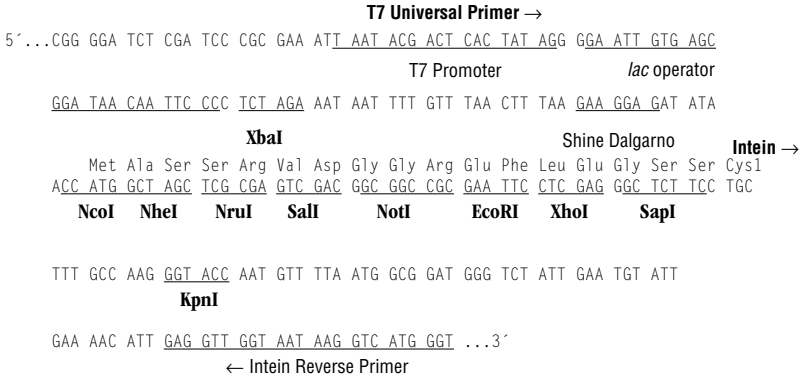

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References:

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. 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., Shao, Y., Paulus, H. Benner, J., Perler F.B. and Xu, M.-Q.(1996). Protein splicing involving the *Saccharomyces, cerevisiae* VMA intein: the steps in the splicing pathway, side reactions leading to protein cleavage, and establishment of an *in vitro* splicing system *J. Biol. Chem*. 271, 22159–22168.
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, 271–281.
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