pTYB4 Vector

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

N6704S 00511111114112

N6704S

10 µg **Lot: 0051111** **Exp: 11/14**

200 µg/ml **Store at –20°C**

Description: pTYB4 is an *E. coli* expression vector (7,474 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 fusion of the N-terminus of the target protein to the intein tag, use pTYB11 (NEB #N6901) or pTYB12 (NEB #N6902).

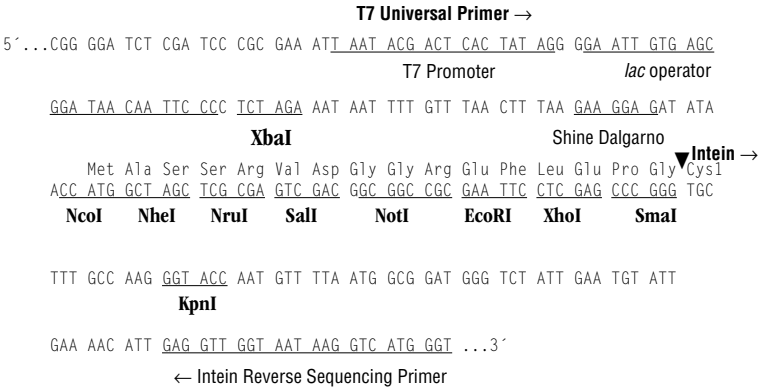
Source: pTYB4 is isolated from an *E. coli* strain (r- m-) by a standard plasmid purification procedure.

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

Features of this vector:

- The NcoI site in the polylinker contains an ATG sequence for translation initiation.
- Use of the SmaI site for cloning will yield a target protein with a single glycine residue added to its C-terminus after cleavage.
- Unique sites indicated in **bold**.
- Expression of the fusion gene is under the control of an IPTG-inducible T7 promoter (4).
- Origin of DNA replication from bacteriophage M13, which allows for the production of single-stranded DNA by helper phage isupernfection of cells bearing the plasmid (M13K07 Helper Phage, NEB #N0315)
- A pBR322 derivative with a ColE1 derivative 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, pTYB3) 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.

Polylinker Region:



(see other side)

CERTIFICATE OF ANALYSIS

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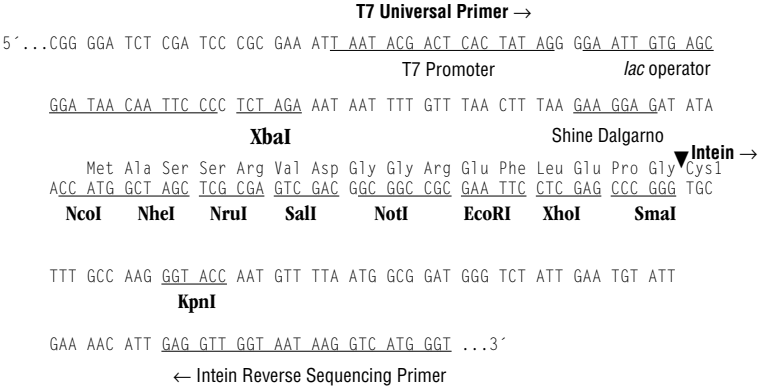

(see other side)

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, 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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