

pTYB21 Vector



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



N6709S 001140217021

N6709S

10 µg Lot: **0011402** Exp: **2/17**
200 µg/ml Store at **-20°C**

Description: pTYB21 is an *E. coli* cloning and expression vector (7514 bp) used in the IMPACT™ Protein Purification System which allows the overexpression of a target protein as a fusion to a self-cleavable affinity tag (1,2). It is a N-terminal fusion vector designed for in-frame insertion of a target gene into the polylinker, downstream of the intein tag (the Sce VMA intein/chitin binding domain, 55 kDa)(3,4). This allows the N-terminus of the target protein to be fused to the intein tag. The self-cleavage activity of the intein allows the release of the target protein from the chitin-bound

intein tag, resulting in a single column purification of the target protein.

This vector can be used in conjunction with a C-terminal fusion vector to test which fusion construction (N-terminal or C-terminal) maximizes the expression and yield of a target protein. For the fusion of the C-terminus of the target protein to the intein tag, use pTXB1 (NEB #N6707), pTXB3 (NEB #N6708), pTYB1 (NEB #N6701), pTYB2 (NEB #N6702), pTYB3 (NEB #N6703) or pTYB4 (NEB #N6704).

Source: pTYB21 is isolated from an *E. coli* strain (r⁻m⁻) by a standard plasmid purification procedure.

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

Features of pTYB21:

Multiple Cloning Sites (MCS):

pTYB21

```
5' ..CAGAACGGAAGAGCTCATATGTCATGGGCGGCCGCGATATCGTCGACGGATCCGAATTCCTGCAGGTAATTAATAA
      Q N G R A H M S M G G R D I V D G S E F P A G N *
```

- The multiple cloning site (MCS) is compatible with the multiple cloning sites of vectors in the pMAL Protein Fusion and Purification System (NEB #E8200) and the *K. lactis* Protein Expression Kit (NEB #E1000).
- When the SapI (or BspQI) site in the MCS is used for cloning the 5' end of the target gene, the N-terminus of the target protein is immediately adjacent to the intein cleavage site. This results in the purification of a target protein without any extra vector-derived residues at its N-terminus. After cloning the target gene in the MCS using SapI, the recognition sequence of SapI is lost; therefore, the vector cannot be recut with SapI. For details, see the IMPACT Manual.
- When NdeI is used for cloning the 5' end of the target gene, extra amino acids (Gly-Arg-Ala-

- His) will be added to the N-terminus of the target protein.
- A stop codon should be included in the reverse primer.
- A pBR322 derivative with a ColE1 replication origin.
- Expression of the fusion gene is under the control of the T7/*lac* promoter and can be induced by IPTG due to the presence of a *lac* gene (5).
- Expression requires an *E. coli* host that carries the T7 RNA Polymerase gene [e.g., T7 Express Competent *E. coli*, NEB #C2566].
- Ampicillin resistance.
- When pTYB21 or pTYB22 is used, a small peptide (15 amino acids, 1.6 kDa) is also cleaved from the intein tag and co-eluted with the target protein. It cannot be detected on a regular SDS-PAGE and can be dialyzed out.
- Origin of DNA replication from the bacteriophage M13 allows for the production

(See other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

of single-stranded DNA by helper phage superinfection of cells bearing the plasmid. M13K07 Helper Phage (NEB #N0315) is available.

- Other IMPACT vectors are available which allow for fusion of a target gene to N- or C-terminus of an intein and a cleavage reaction which can be induced by thiol reagent or temperature/pH shift.
- Intein Forward Primer (NEB #S1263) and T7 Terminator Reverse Primer (NEB #S1271) are available for sequencing the target gene.

References:

1. 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.
2. Chong, S., Mersha, F.B., Comb, D.G., Scott, M. E., Landry, D., Vence, L.M., Perler, F.B.,

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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.

3. Chong, S., Williams, K.S., Wotkowicz, C., and Xu, M.Q. (1998). Modulation of protein splicing of the *Saccharomyces cerevisiae* vacuolar membrane ATPase intein. *J. Biol. Chem* 273,10567–77.
4. 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.
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

2. 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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Use of the host cells that contain the copy of the T7 gene 1, the gene for T7 RNA polymerase with any other vector(s).

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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 agreements may be obtained from the Office of Technology Transfer, Brookhaven National Laboratory, Building 475D, P.O. Box 5000, Upton, New York 11973-5000; Tel. 631-344-7134, Fax: 631-344-3729.

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Note: *E. coli* strain ER2566 is only available to purchasers of the IMPACT™ System or replacement vectors.

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

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