pTYB21 Vector

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

N6709S 001130416041

N6709S

10 µg **Lot: 0011304** **Exp: 4/16**

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 conjuction 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:

- 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 *lacI* 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

Multiple Cloning Sites (MCS):

pTYB21

```
          Intein      SapI       NdeI       NcoI       NotI       EcoRV      SalI       BamHI      EcoRI      SbfI/PstI
5´...CAGAACGGAAGAGCTCATATGTCCATGGGCGGCCGCGATATCGTCGACGGATCCGAATTCCCTGCAGGTAATTAAATAA
     Q  N  G  R  A  H  M  S  M  G  G  R  D  I  V  D  G  S  E  F  P  A  G  N  *
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(See other side)

CERTIFICATE OF ANALYSIS

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Multiple Cloning Sites (MCS):

pTYB21

Intein SapI NdeI NcoI NotI EcoRV SalI BamHI EcoRI SbfI/PstI
5´...CAGAACGGAAGAGCTCATATGTCCATGGGCGGCCGCGATATCGTCGACGGATCCGAATTCCCTGCAGGTAATTAAATAA
Q N G R A H M S M G G R D I V D G S E F P A G N *

(See other side)

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., 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 www.neb.com.

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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

- Origin of DNA replication from the bacteriophage M13 allows for the production 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.
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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) 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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