pTYB12 Vector

Catalog #	Size	Concentration	Price	Qty	
N6902S	10 µg	200 µg/ml	$79.00	1	ADD TO CART

Prices are in US dollars and valid only for US orders.

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Description:
pTYB12 is an *E. coli* cloning and expression vector (7417 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 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 pTYB2 (NEB #N6702S) 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 pTYB1 (NEB #N6701S), pTYB2 (NEB #N6702S), pTYB3 (NEB #N6703S), or pTYB4 (NEB #N6704S).

Source:
pTYB12 is isolated from an *E. coli* strain (r-m-) by a standard plasmid purification procedure.

Advantages:

- The sites in the polylinker region are identical to or compatible with (i.e. SpeI of pTYB12 and NheI of pTYB2) those of pTYB2 (NEB #N6702S). This allows the same amplified target gene fragment 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.
- Other IMPACT vectors are available which allow for fusion of a target gene to N- or C- terminus of an intein. The cleavage reaction can be induced by thiol reagent or temperature/pH shift.
- After the cleavage of the intein tag, a target protein is obtained with extra residue(s) added to N-terminus. For instance, cloning the 5´ end of a target gene using NdeI site in pTYB12 adds extra three residues (Ala-Gly-His) to the N-terminus of the target protein.
- A pBR322 derivative with a ColE1 replication origin.
- Expression of the fusion gene is under the control of the T7 promoter and can be induced by IPTG due to the presence of a *lac*I gene (5).
- Expression requires an *E. coli* host that carries the T7 RNA Polymerase gene [e.g., ER2566 or BL21(DE3) and derivatives].
- Ampicillin resistence.
- 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 #N0315S) is available.
- Intein Forward Primer (NEB #S1263S) and T7 Terminator Reverse Primer (NEB #S1271S) are available for sequencing the target gene.

Concentration:
200 µg/ml

Storage Conditions

Storage Conditions:
10 mM Tris-HCl
1 mM EDTA
pH 8.0 @ 25°C

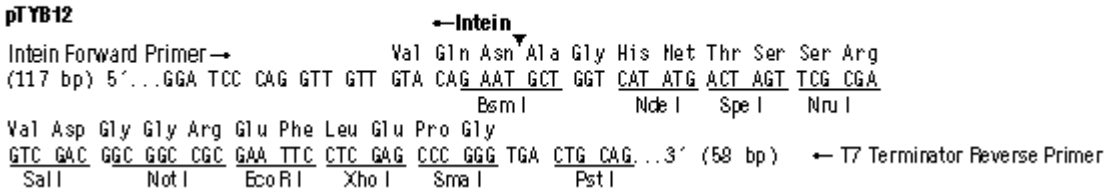
Storage Temperature:
-20°C

Notes

General notes:

1. Product ER2566 is only available to purchasers of the IMPACT™ System or replacement vectors.

Multiple Cloning Sites (MCS):



References

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