**IMPACT™-CN System**

Protein Purification System Now Featuring Fusion to C- or N- Terminus of the Target Protein

#E6900S .................. $420 (USA)

**Description**

IMPACT™ (Intein Mediated Purification with an Affinity Chitin-binding Tag) is a novel protein purification system which utilizes the inducible self-cleavage activity of a protein splicing element (termed intein) to separate the target protein from the affinity tag. It distinguishes itself from all other purification systems by its ability to purify, in a single chromatographic step, a native recombinant protein without the use of a protease. A target protein is fused to a tag consisting of the intein and the chitin binding domain which allows affinity purification of the fusion precursor on a chitin column. In the presence of thiols such as DTT, β-mercaptoethanol or cysteine, the intein undergoes specific self-cleavage which releases the target protein from the chitin-bound intein tag resulting in a single-column purification of the target protein (Figure 1).

Use of pTYB2 or pTYB12 yields a target protein with extra residue(s) added to its C-terminus or N-terminus, respectively, after the cleavage of the intein tag; when pTYB11 and pTYB12 are used, a small peptide (1.2 kDa) is also cleaved from the intein tag and co-eluted with the target protein. It cannot be detected on a regular SDS-PAGE gel but can be separated from the target protein by dialysis. pTYB1 and pTYB2 use ATG of the target gene to be cloned adjacent to the cleavage site of the intein tag; this results in the purification of a target protein without any extra non-native residues attached to its terminus. pTYB2 and pTYB12 contain the same or compatible restriction sites in the multiple cloning region. This allows fusion of the intein tag to either termini of the same amplified target gene.

![Figure 1: Schematic illustration of the IMPACT-CN System.](image)

The IMPACT-CN system contains expression vectors (pTYB vectors) which allow fusion of the cleavable intein tag to either the C-terminus or N-terminus of the target protein. This flexibility in fusion protein construction maximizes the probability of successful expression and purification of a target protein. To allow the cloning of the same amplified target gene in either fusion construction, the same or compatible restriction sites are designed in the multiple cloning region of pTYB12 and pTYB12 vectors. pTYB1 and pTYB11 vectors, on the other hand, allow the cloning of a target gene immediately adjacent to the intein cleavage site. This results in the purification of a native target protein without any vector-derived extra residues after the cleavage.

**Advantages**

- **Flexibility**—allows fusion to either the C-terminus or N-terminus of the target protein
- **Yields proteins with native sequence**
- **Release of fusion partner without the use of proteases**
- **One-step affinity purification—no additional steps to remove affinity tag**
- **T7 Promoter for higher levels of expression**
- **Tight transcriptional control**
- **The ability to label the C-terminus of the target protein**

**The IMPACT Kit Includes:**

- Vector DNA (4 vectors) (10 µg each)
- E. coli strain ER2566 (0.2 ml)
- Chitin Beads (20 ml)
- pMYB5 Control Plasmid (10 µg)
- Anti-Chitin Binding Domain Serum
- Sequencing Primers (4 primers) (200 picomoles of each)
- 1,4-dithiothreitol (DTT), 1 M (5 ml)
- Blue Loading Buffer (1 ml)
- Detailed Instruction Manual

**Cloning Vectors:**

- The pTYB vectors are used for cloning and expression of recombinant proteins in E. coli (Figure 2). pTYB1 and pTYB2 are C-terminal fusion vectors in which the C-terminus of the target protein is fused to the intein tag. pTYB11 and pTYB12 are N-terminal fusion vectors in which the N-terminus of the target protein is fused to the intein tag; when pTYB11 and pTYB12 are used, a small peptide (1.2 kDa) is also cleaved from the intein tag and co-eluted with the target protein. It cannot be detected on a regular SDS-PAGE gel but can be separated from the target protein by dialysis. pTYB1 and pTYB2 use ATG of the Nde I site in the multiple cloning region for translation initiation. Both pTYB1 and pTYB11 contain a Sap I cloning site which allows the target gene to be cloned adjacent to the cleavage site of the intein tag; this results in the purification of a target protein without any extra non-native residues attached to its terminus. pTYB2 and pTYB12 contain the same or compatible restriction sites in the multiple cloning region. This allows fusion of the intein tag to either termini of the same amplified target gene.

Use of pTYB2 or pTYB12 yields a target protein with extra residue(s) added to its C-terminus or N-terminus, respectively, after the cleavage of the intein tag. For instance, cloning the 3’ end of a target gene using the Sma I site in pTYB2 adds an extra glycine residue to the C-terminus of the target protein. Likewise, cloning the 5’ end of a target gene using the Nde I site in pTYB12 adds four extra residues (Ala-Gly-His-Met) to the N-terminus of the target protein.

The pTYB vectors use a T7 promoter and the lac I gene to provide stringent control of the fusion gene expression. Binding of the lac repressor to the lac operator sequence immediately downstream of the T7 promoter suppresses basal expression of the fusion gene in the absence of IPTG induction. The four tandem copies of the E.coli transcription terminator (rntB T1) placed upstream of the promoter minimize background transcription. The vectors also contain the origin of DNA replication from bacteriophage M13, which allows for the production of single-stranded DNA by helper phage (M13KO7 Helper Phage, NEB #N0315S) superinfection of cells bearing the plasmid. pTYB vectors carry the Amp′ gene marker (the bla gene), which conveys ampicillin resistance to the host strain.

(see other side)
Figure 3: Expression and purification of the E. coli maltose-binding protein (MBP) using the IMPACT-CN System.

- **Figure 2**:  Val Gln Asn Ala Gly His Met Thr Ser Ser Arg

  - **Figure 1**: Multiple cloning sites in pTYB vectors.

  - **Figure 3**: Companion Products

**Sequencing Primers**: Three primers (200 picomoles of each) are included for sequencing the target gene cloned in the multiple cloning region of the pTYB vectors. The T7 Universal Primer and Intein Reverse Primer are used for sequencing a target gene cloned in the C-terminal fusion vectors (pTYB1 and pTYB2). The intein forward primer is complementary to the intein sequence 117-141 nucleotides upstream from the intein cleavage site and used for sequencing a target gene cloned in the N-terminal fusion vectors (pTYB11 and pTYB12).

**Chitin Beads**: An affinity matrix used for the isolation of the fusion precursor containing the target protein. 20 ml of chitin beads (~50-100 µm in size) are supplied as a 38 ml slurry in 20% ethanol. The binding capacity, which has been tested using the control vector pMYBS, is 2 mg of eluted MBP protein per ml of chitin beads. Chitin Beads should be stored at 4°C. Temporary storage at ~20°C will not affect the binding capacity.

**References**