pTXB1

Location: Expresión 13
Resistance: Ampicillin 100 µg/mL

Description pTXB1 vector :
pTXB1 is an *E. coli* expression vector in the IMPACT™ Kit (NEB #E6901). It is designed for the in-frame insertion of a target gene into the polylinker upstream of the *Mxe* intein/chitin binding domain (27 kDa). The fusion protein is bound to chitin beads and the thiol-induced cleavage activity of the intein releases the target protein. pTXB vectors are recommended for use in intein-mediated protein ligation and C-terminal labeling . This double stranded vector is 6,706 base pairs in length.

Genotype of *E coli* strain BL21(DE3) pLysS : F– *ompT hsdS*(rB– mB–) *gal dcm* λ(DE3) pLysS (Camr)(λ(DE3): *lac*I, *lac*UV5-T7 gene 1, *ind*1, *sam*7, *nin*5)

Polylinker Region:

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T7 Universal Primer →

5´...CGG GGA TCT CGA TCC CGC GAA ATT AAT ACG ACT CAC TAT AGG GGA ATT GTG AGC

T7 Promoter *lac* operator

GGA TAA CAA TTC CCC TCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA GAT ATA

XbaI **ShineDalgarno**

Intein →

Met Ala Ser Ser Arg Val Asp Gly Gly Arg Glu Phe Leu Glu Gly Ser Ser Cysl
CAT ATG GCT AGC TCG CGA GTC GAC GGC GGC CGC GAA TTC CTC GAG GGC TCT TCC TGC

NdeI NheI NruI SalI NotI EcoRI XhoI SapI

ATC ACG GGA GAT GCA CTA GTT GCC CTA CCC GAG GGC GAG TCG GTA

SpeI

CGC ATC GCC GAC ATC GTG CCG ...3´

Features

- The NdeI site in the polylinker contains an ATG sequence for translation initiation. Unique sites are indicated in bold. SalI site is not unique.
- The SapI site should be used for cloning of the 3´ end of the insert. Use of the SapI site allows cloning of the target protein adjacent to the intein, resulting in cleavage of the target protein without any additional amino acids at its C-terminus.
- Expression of the fusion gene is under the control of an IPTG-inducible T7 promoter (6).
- A pBR322 derivative with a ColE1replication origin.
- Origin of DNA replication from bacteriophage M13, which allows for the production of single-stranded DNA by helper phage superinfection of cells bearing the plasmid (M13K07 helper phage, NEB #N0315).
- Ampicillin resistance.

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- 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 vector pTXB3 (NEB #N6708) contains an NcoI site in place of NdeI.
- 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.

Simplified Expression and Purification Protocol:

1. Transformation: Transform the plasmid bearing the target gene into competent T7 Express or competent cells prepared from ER2566.
2. Cell Culture: Inoculate a freshly grown colony in LB medium containing 100 µg/ml ampicillin and grow the cells at 37°C. When the OD600 reaches 0.5, induce protein expression by adding IPTG to a final concentration of 0.4 mM, and incubate at 30–37°C.
3. Column Preparation: Equilibrate a chitin column (20 ml slurry for 1 liter culture) with 10 column volumes of Column Buffer [20 mM Tris-HCl (pH 8.5), 500 mM NaCl].
4. Cell Harvest: Centrifuge cell culture at 5,000 x g for 15 minutes at 4°C. Discard supernatant. Resuspend cell pellet in column buffer.
5. Loading: Break cells by sonication in Column Buffer, and centrifuge at 15,000 g for 30 minutes at 4°C. Slowly load the clarified lysate onto the chitin column (0.5–1.0 ml/minute).
6. Washing: Wash the column with at least 20 bed volumes of Column Buffer to thoroughly remove the unbound proteins (up to 2.0 ml/minute).
7. Adding Thiols: Quickly wash the column with 3 column volumes of Cleavage Buffer [Column buffer containing 50 mM DTT (for purification) or 50 mM MESNA (for IPL)].
8. On-column Cleavage: Stop the flow and incubate the column at 4°C–23°C for 16–40 hours. The temperature and duration of the cleavage reaction are dependent on the on-column cleavage efficiency which can be checked by analyzing samples of chitin resin before and after cleavage.
9. Elution: Elute the target protein with Column Buffer by continuing the column flow.
10. Dialysis: Dialyze the target protein in to an appropriate storage buffer; this will also remove the excess thiol reagent used in the Cleavage Buffer and the co-eluted small peptide (when using pTYB21).
11. Cleavage: To examine cleavage efficiency remove 100 µl of chitin resin and mix with 50 µl of 3X SDS Sample Buffer. After boiling for 5 minutes, analyze the supernatant on a Coomassie stained SDS-PAGE gel to determine the cleavage efficiency.
12. Regeneration of Chitin Resin: Wash the column with 3 bed volumes of the 0.3 M NaOH (Stripping Solution). Allow the resin to soak for 30 minutes and wash the resin with an additional 7 bed volumes of 0.3 M NaOH. Wash with 20 bed volumes of water, followed by 5 bed volumes of column buffer.

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Media and Solutions:

The following are suggested media for cell culture, cell lysis and protein purification. They can be modified according to the specific properties of the target protein.

LB broth (per liter)

10 g tryptone
5 g yeast extract
10 g NaCl
Adjust pH to 7.0 with NaOH

Column Buffer

20 mM Na-HEPES (or Tris-HCl), pH 8.5
500 mM NaCl (or 50–1,000 mM NaCl)
1 mM EDTA (optional)
Nonionic detergents (0.1–0.5% Triton X-100 or 0.1–0.2% Tween 20) and protease inhibitors [e.g., PMSF (20 µM)] can also be included. For a target protein sensitive to oxidation, 1 mM of TCEP [tris-(2-carboxyethyl)phosphine] or TCCP [tris-(2-cyanoethyl)phosphine] may be used.

Cleavage Buffer

20 mM Na-HEPES (or Tris-HCl), pH 8.5
500 mM NaCl (or 50–1,000 mM NaCl)
50 mM DTT or β-mercaptoethanol or cysteine*
1 mM EDTA (optional)

Stripping Solution

0.3 M NaOH

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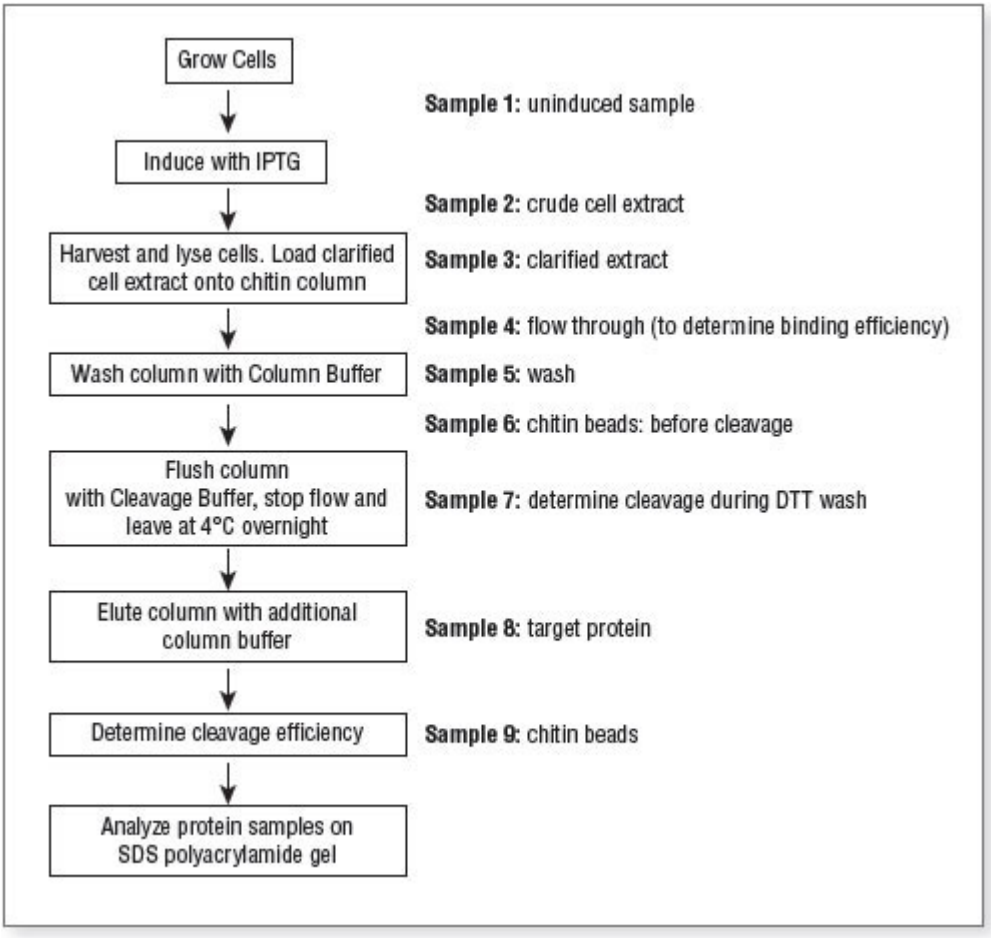

Flow chart for Protein Expression and Purification using the IMPACT System. Sample collection for analysis by SDS-PAGE is indicated.

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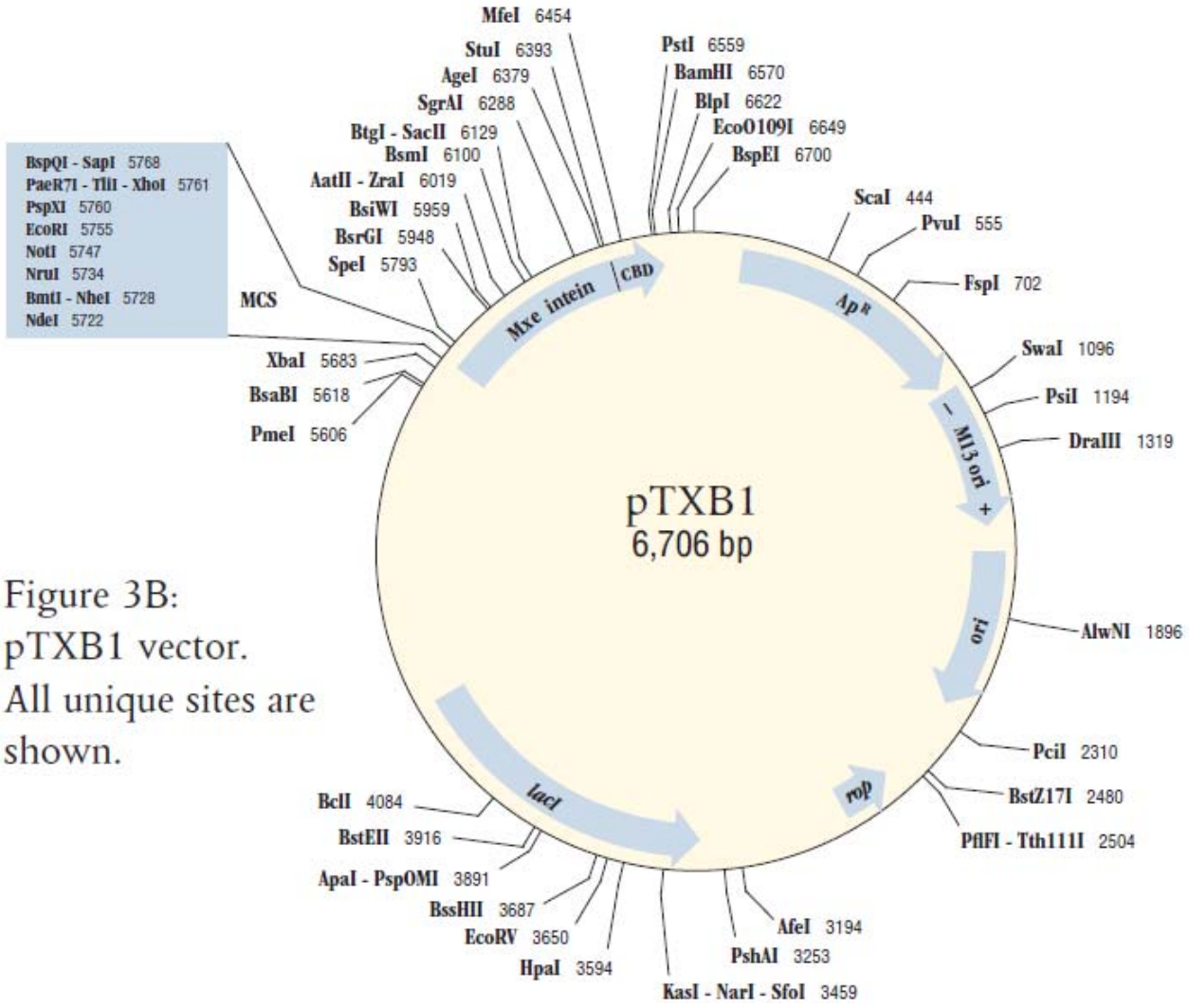

Figure 3B:
pTXB1 vector.
All unique sites are shown.

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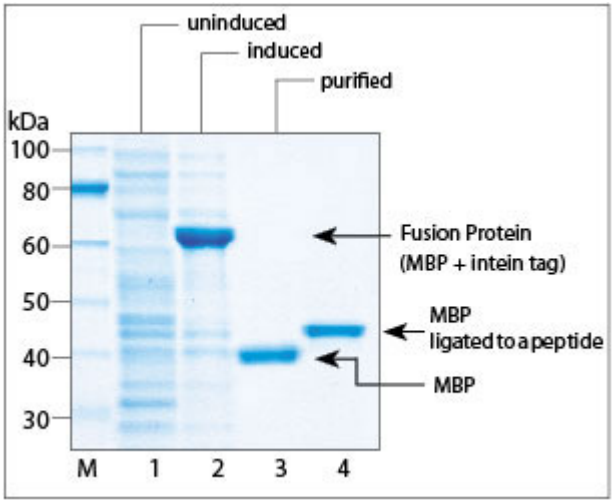

Purification of Maltose Binding Protein (MBP) in a single affinity purification step: Lane 1: uninduced cell extract. Lane 2: induced cell extract showing expressed fusion protein. Lane 3: MBP fractions eluted after inducing cleavage overnight at 4°C. Lane 4: MBP ligated to a peptide containing an N-terminal cysteine. Marker M is the Protein Ladder

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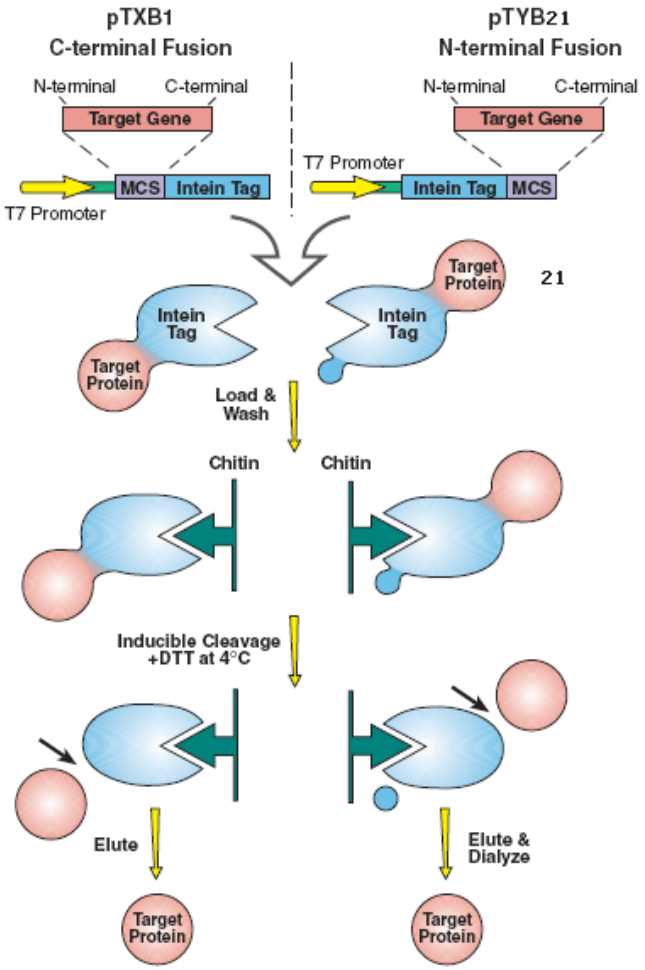

Schematic of the IMPACT System.

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Intein-mediated Protein Ligation (IPL).

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