

Product Name : pET Expression vector pETIA
Code No. : DV215
Size : 15 µg (lyophilized plasmid contains salt of TE buffer)
Storage : Store at -20°C

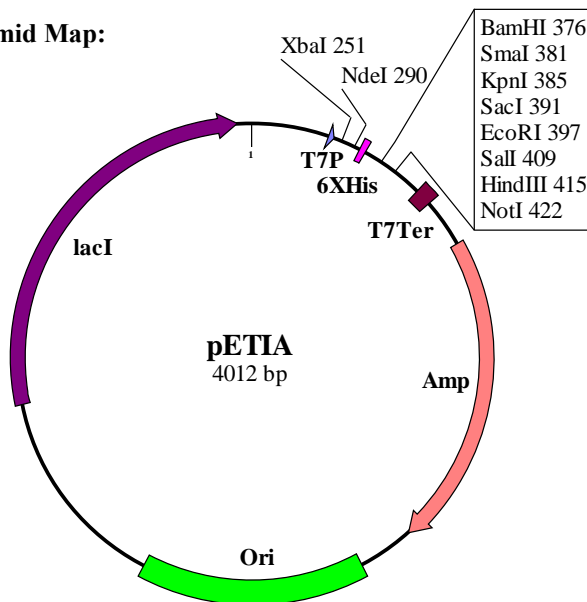
日本語データシート



Product Description:

pETIA is a medium copy number, ampicillin resistant, stringent controllable T7 bacterial expression vector. The T7 expression system is one of the strongest expression systems and has been widely used with a coupling of BL21 (DE3) *E. coli* cell. T7 RNA polymerase gene is integrated in a genome of BL21(DE3) under control of lacUV5 promoter. Upon addition of isopropyl-1-thio-β-D-galactopyranoside (IPTG), T7 RNA polymerase is expressed in the BL21(DE3) cells harboring pETIA vector, and it induces a high-level protein expression from T7 promoter of pETIA. The pETIA has a lacI gene, which represses T7 RNA polymerase gene in the absence of IPTG. The regulation with lac repressor is beneficial to repress a basal level protein expression and to maintain a recombinant plasmid in BL21 (DE3) cell.

| Plasmid Map:



| | |
|--------------------------|-----------|
| T7 promoter : | 213-229 |
| T7 transcription start : | 230 |
| His Tag : | 305-322 |
| T7 terminator : | 498-545 |
| lacI : | 2891-3973 |
| pMB1 ori : | 1695-2314 |
| Amp (bla) : | 680-1540 |

| Reconstitution : Resuspend the lyophilized pETIA with 15 µl of sterile water to make 1 µg/µl plasmid in 1 × TE buffer. After reconstitution, store at - 20°C

| Features of T7 expression vectors

BioDynamics Laboratory Inc. provides 6 kinds of T7 expression vectors, pETUA, pETBA, pETIA, pETUK, pETBK, and pETIK. These vectors have the same multicloning site and specific feature of each vector is below:

| | Plasmid copy number | Replicon | Antibiotic resistance | Feature and recommendation |
|-------|---------------------|----------|-----------------------|---|
| pETUA | high copy | pUC | ampicillin | for non-toxic protein expression |
| pETBA | medium copy | pMB1 | ampicillin | general expression |
| pETIA | medium copy | pMB1 | ampicillin | stringent regulation with lac repressor |
| pETUK | high copy | pUC | kanamycin | for non-toxic protein expression |
| pETBK | medium copy | pMB1 | kanamycin | general expression |
| pETIK | medium copy | pMB1 | kanamycin | stringent regulation with lac repressor |

Product Usage

| Cloning of a gene to pETIA:

Below is the multiple cloning site of pETIA. To express a recombinant protein correctly, it is necessary to clone the gene of interest in frame with an N-terminal peptide of pETIA. The start codon of pETIA is boxed ATG in the below figure. Digest pETIA completely with appropriate restriction enzyme(s) to form DNA ends which can be ligated to the gene of interest. If only one restriction enzyme is used, dephosphorylation of a vector is often performed. Ligation of processed pETIA and the gene of interest can be performed by the standard procedure. The following transformation procedure should be done with non-expression hosts such as DH5 α or JM109. Recombinant plasmids derived from pETIA are selected by colony-PCR, enzyme digestion of prepared plasmids, or other methods. Sequencing of cloning portion and an insert region on the obtained plasmid is recommended to determine the correct recombinant plasmids for expression experiments.

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                T7 promoter                                XbaI
GATCCCGCGA AATTAATACG ACTCACTATA GGGAGACCAC AACGGTTTCC CTCTAGAAAT 259
AspProAlaL ysLeuIleAr gLeuThrIle GlyArgProG lnArgPhePr oSerArgAsn
                NdeI                                6xHis
AATTTTGTTT AACTTTAAGA AGGAGATATA CATATGCGGG GTTCTCATCA TCATCATCAT 319
AsnPheVal* **Leu***Gl uGlyAspIle HisMetArgG lySerHisHi sHisHisHis
                EK                                BamHI
CATGGTATGG CTAGCATGAC TGGTGGACAG CAAATGGGTC GGGACGATGA CGATAAGGAT 379
HisGlyMetA laSerMetTh rGlyGlyGln GlnMetGlyA rgAspAspAs pAspLysAsp
                KpnI                EcoRI                SalI                NotI                ↑
CCCCGGGTAC CGAGCTCGAA TTCGATTCG TCGACAAGCT TAGCGGCCGC CGTTTAAATCC 439
                SmaI                SacI                                HindIII
ProArgValP roSerSerAs nSerIleSer SerThrSerL euAlaAlaAl aVal***Ser

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EK: Enterokinase recognition sequence (AspAspAspAspLys↓)

ATG: start codon

TAA: stop codon

| pETIA Sequence

DNA sequences are available on our web site.



<https://www.biodynamics.co.jp/products/ex-pack/>

| **Expression:**

1. Following transformation, pick a colony and inoculate it into 3 mL of LB medium containing the appropriate antibiotic with shaking at 37°C, overnight.
2. The next morning, transfer 0.5 mL of the overnight culture to a new 10 mL of LB medium containing the appropriate antibiotic to select the expression plasmid. Grow the culture with shaking at 37°C until the OD₆₀₀ reaches 0.5 (approximately 2 hrs but this depended on the expression plasmids).

When using BL21(DE3)pLys, chloramphenicol is not usually required in the short-period culture.

3. When the OD₆₀₀ reaches 0.5, transfer an aliquot (e.g., 1 mL) of the culture to a new centrifuge tube and centrifuge it to harvest cells. Store the cells at -80°C until analysis.

Add IPTG to a final concentration of 1 mM to the rest of the culture and grow the culture with shaking at 37°C for 3 hours.

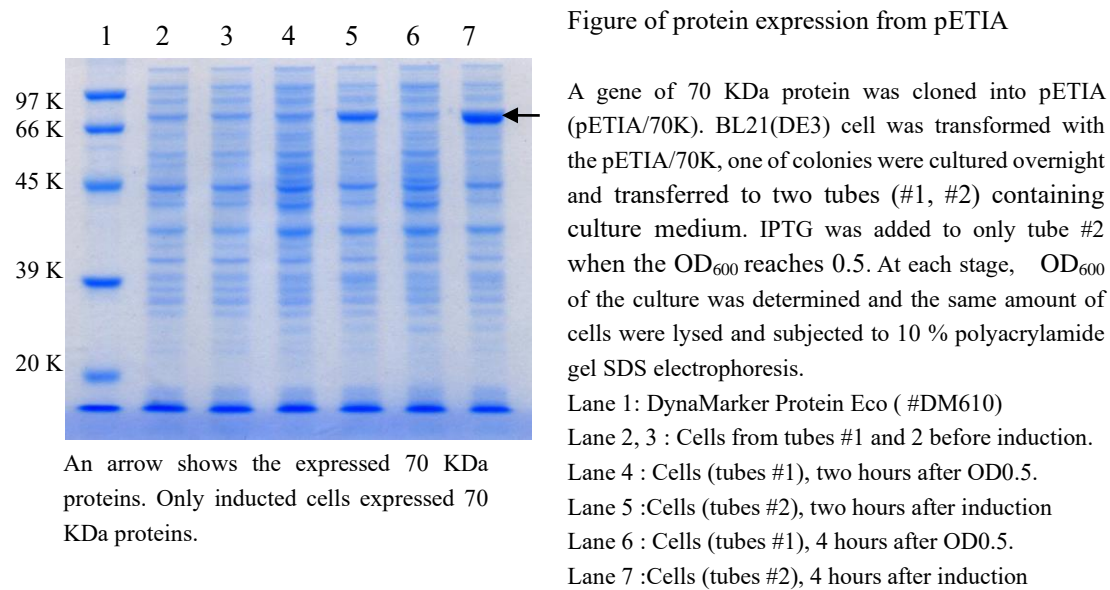
The IPTG concentration and induction time are general values. It is recommended to determine the optimal condition for the target gene expression.

4. After the induction, harvest the cells. To analyze the expression, before harvesting the cells, transfer an aliquot of the culture (e.g., 1 mL) and centrifuge it to precipitate the cells.

| **Analysis:**

1. Suspend the precipitated cells (from the 1 mL culture) in 200 µl of 1× PBS buffer.
2. Mix an aliquot of the suspension (e.g., 100 µl) with an equal volume of 2 × SDS sample buffer.
3. Heat the mixture at 85°C for 5 min, then centrifuge at 10,000 g for 10 min. Subject the supernatant (e.g., 5-25 µl) to SDS-PAGE. Western blot will help analyzing the expression of the target protein.

- 2 × SDS sample buffer : 2 % sodium dodecyl sulfate, 5 % 2-mercaptoethanol, 20 % glycerol, 0.02 % BPB, 62.5 mM Tris-HCl, pH6.8
- 1× PBS buffer.: 20 mM sodium phosphate, 150 mM sodium chloride, pH7.4



● Notes for expression:

1. As the T7 expression method is a high-level protein expression system, some basal level expression of the target protein will occur in uninduced cells. This is likely problematic in cases in which the target protein is toxic to *E. coli* cells. In this case, it may be necessary to decrease the basal level expression as follows:

- a) Use a lower-copy number T7 expression vector, pETBA, pETBK, but not pETUA, pETUK
- b) Use a stringent regulated expression vector, pETIA, pETIK.
- c) Use liquid medium and agar plates supplemented with glucose (0.5 -1 %).
Glucose is known to decrease a basal expression from *lacUV5* promoter².
- d) Use BL21(DE3)pLysS strain but not BL21(DE3) strain.

The T7 Lysozyme encoded in a pLysS plasmid reduces the basal level of T7 RNA polymerase Expression³. This leads to suppression of the basal level expression of the target protein.

2. When expressing proteins in BL21(DE3) cells, if it takes a longer time (5 hrs or more) to reach 0.5 at OD₆₀₀ after inoculating the overnight culture (0.5 ml) to a new LB medium (10 ml), the expressed protein is likely toxic to *E. coli* cells.

3. When BL21(DE3) cells lyse after induction with IPTG, the expressed protein is likely toxic to *E. coli* cells.

Reference:

- 1) Studier, F.W. and Moffatt, B.A., *J. Mol. Biol.* 189 (1986) 113–130.
- 2) Moffatt, B.A. and Studier, F.W., *Cell* 49 (1987) 221-227
- 3) Pan, S. and Malcom, B.A., *BioTechniques* 29 (2000), 1234–1238

General reference in this Product Information

Sambrook, J. and Russell, D.W. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Related Products:

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|-------|--|
| DS255 | DynaCompetent Cells Zip BL21(DE3) |
| DS260 | DynaCompetent Cells BL21(DE3)pLysS |
| DS230 | DynaCompetent Cells JetGiga DH5 α |
| DS520 | AllView PAGE Buffer |
| DM660 | DynaMarker Protein MultiColor Stable II |
| DS850 | |
| DS860 | ONEPot Immunoassay Kit <OpenGUS Method> |