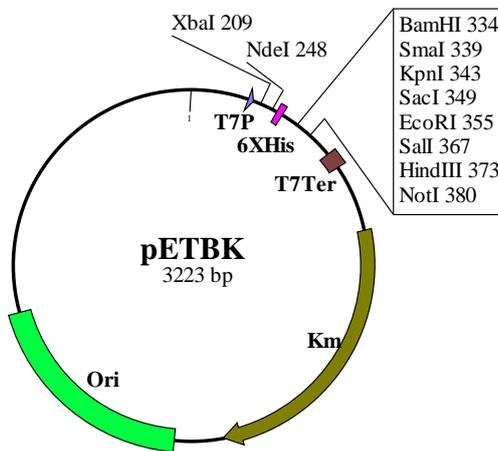


Product Name : pET Expression vector pETBK
Code No. : DV230
Size : 15 µg (lyophilized plasmid contains salt of TE buffer)
Storage : Store at -20°C

Product Description:

pETBK is a medium copy number, kanamycin resistant, 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* strain. 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 pETBK vector, and it induces a high-level protein expression from T7 promoter of pETBK. BioDynamics Laboratory Inc. offers several kinds of T7 bacterial expression vectors. Among them, pETBK is standard kanamycin-resistant vector for a high level expression of proteins.

| Plasmid Map:



T7 promoter : 171-187
 T7 transcription start : 188
 His Tag : 263-280
 T7 terminator : 456-503
 pMB1 ori : 1660-2279
 Km : 703-1518

| Reconstitution : Resuspend the lyophilized pETBK 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 pETBK:

Below is the multiple cloning site of pETBK. To express a recombinant protein correctly, it is necessary to clone the gene of interest in frame with an N-terminal peptide of pETBK. The start codon of pETBK is boxed ATG in the below figure. Digest pETBK 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 pETBK 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. In the transformation, recombinant cells should be selected on LB agar plates containing 15-25 μ g/ml of kanamycin., because higher concentration of kanamycin often retarded cell growth on the agar plates. Recombinant plasmids derived from pETBK 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.

	T7 promoter		XbaI			
GATCCCGCGA	AATTAATACG	ACTCACTATA	GGGAGACCAC	AACGGTTTCC	CTCTAGAAAT	217
AspProAlaL	ysLeuIleAr	gLeuThrIle	GlyArgProG	lnArgPhePr	oSerArgAsn	
			NdeI		6 \times His	
AATTTTGTTT	AACTTTAAGA	AGGAGATATA	CATATGCGGG	GTTCTCATCA	TCATCATCAT	277
AsnPheVal*	**Leu***Gl	uGlyAspIle	HisMetArgG	lySerHisHi	sHisHisHis	
				EK	BamHI	
CATGGTATGG	CTAGCATGAC	TGGTGGACAG	CAAATGGGTC	GGGACGATGA	CGATAAGGAT	337
HisGlyMetA	laSerMetTh	rGlyGlyGln	GlnMetGlyA	rgAspAspAs	pAspLysAsp	
	KpnI	EcoRI	SalI	NotI	↑	
CCCCGGGTAC	CGAGCTCGAA	TTCGATTCG	TCGACAAGCT	TAGCGGCCGC	CGTTTAAATCC	397
	SmaI	SacI	HindIII			
ProArgValP	roSerSerAs	nSerIleSer	SerThrSerL	euAlaAlaAl	aVal***Ser	

EK: Enterokinase recognition sequence (AspAspAspAspLys↓)

ATG: start codon

TAA: stop codon

| pETBK 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.

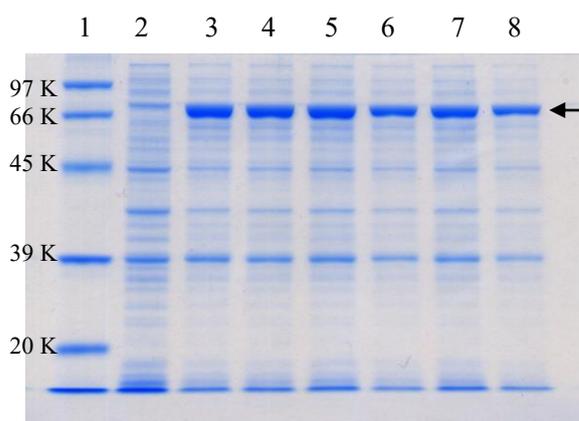
The IPTG concentration and induction time are general values. It is recommended to determine the optimal condition for the target gene expression.

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



An arrow shows the expressed 65 KDa proteins.

Figure of protein expression from pETBK

A gene of 65 KDa protein was cloned into pETBK (pETBK/65K). BL21(DE3) cell was transformed with the pETBK/65K, six colonies were picked and followed the “Protein Expression Procedure” as above. After induction, aliquot of the cells from each culture was subjected to 10 % polyacrylamide gel SDS electrophoresis. The gel was stained with Quick Blue Protein Staining Solution (BioDynamics Laboratory Inc. #DS500).

Lane 1: DynaMarker Protein Eco (#DM610)

Lane 2 : BL21(DE3) harboring pETBK but not pETBK/65K

Lane 3-8 : BL21(DE3) cells, clones 1-6

● 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:

DS255	DynaCompetent Cells Zip BL21(DE3)
DS260	DynaCompetent Cells BL21(DE3)pLysS
DS230	DynaCompetent Cells JetGiga DH5α
DS520	AllView PAGE Buffer
DM660	DynaMarker Protein MultiColor Stable II
DS850	ONEPot Immunoassay Kit <OpenGUS Method>
DS860	