

PRODUCT INFORMATION

Product Name : pET Expression pack (pETIA)

Code No. : DS255IA

Kit Component :

Component	Code No.	Contents
pET Expression vector pETIA	DV215	15 µg (lyophilized plasmid contains salt of TE buffer)
Zip Competent Cell BL21(DE3)	DS255	3 tubes (100 µL/tube), transformation efficiency: 2×10^6 cfu/µg (pUC19)

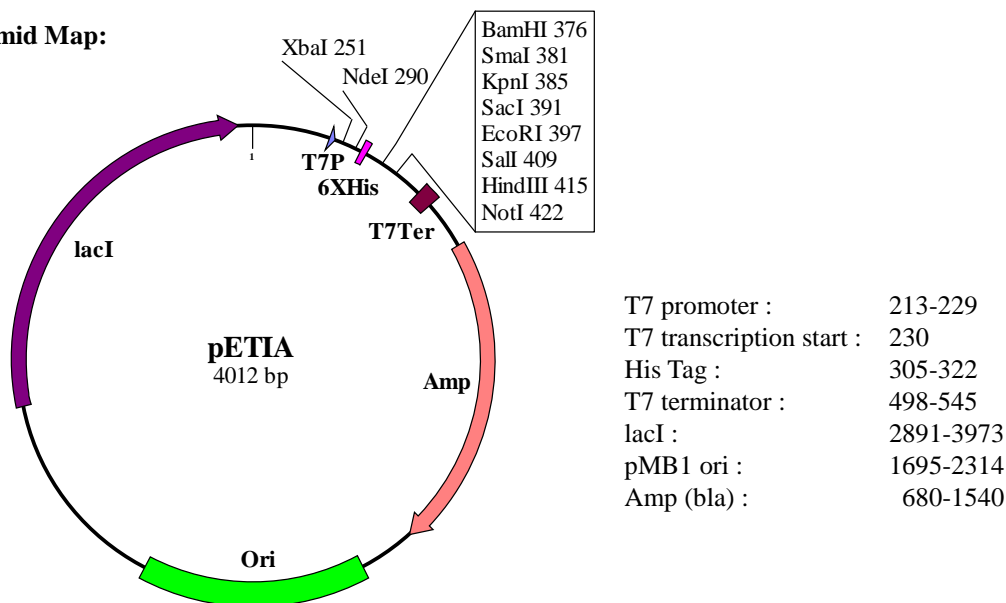
Storage : Store at -80°C

Product Description:

1) pET Expression vector pETIA

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:



| 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

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| 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

| pETIA Sequence

GTTTGACAGC	TTATCATCGA	CTGCACGGTG	CACCAATGCT	TCTGGCGTCA	GGCAGCCATC	60
GGAAGCTGTG	GTATGGCTGT	GCAGGTCGTA	AATCACTGCA	TAATTCGTGT	CGCTCAAGGC	120
GCACTCCCGT	TCTGGATAAT	GTTTTTTGCG	CCGACATCAT	AACGGTTCCTG	GCAAATATTC	180
TGAAATGAGC	TGAGATCTCG	ATCCCGCGAA	ATTAATACGA	CTCACTATAG	GGAGACCACA	240
ACGGTTTCCC	TCTAGAAATA	ATTTTGTTTA	ACTTTAAGAA	GGAGATATAC	ATATGCGGGG	300
TTCTCATCAT	CATCATCATC	ATGGTATGGC	TAGCATGACT	GGTGGACAGC	AAATGGGTCG	360
GGACGATGAC	GATAAGGATC	CCCGGGTACC	GAGCTCGAAT	TCGATTTTCGT	CGACAAGCTT	420
AGCGGCCGCC	GTTTAATCCG	GCTGCTAACA	AAGCCCAGAA	GGAAGCTGAG	TTGGCTGCTG	480
CCACCGCTGA	GCAATAACTA	GCATAACCCC	TTGGGGCCCTC	TAAACGGGTC	TTGAGGGGTT	540
TTTTTGCTGAA	AGGAGGAACT	ATATCCGGAT	GCGTTTCTAC	AAACTCTTTT	GTTTATTTTT	600
CTAAATACAT	TCAAATATGT	ATCCGCTCAT	GAGACAATAA	CCCTGATAAA	TGCTTCAATA	660
ATATTGAAAA	AGGAAGAGTA	TGAGTATTCA	ACATTTCCGT	GTCGCCCTTA	TTCCCTTTTT	720
TGCGGCATTT	TGCCTTCCTG	TTTTTGCTCA	CCCAGAAACG	CTGGTGAAAG	TAAAAGATGC	780
TGAAGATCAG	TTGGGTGCAC	GAGTGGGTTA	CATCGAACTG	GATCTCAACA	GCGGTAAGAT	840
CCTTGAGAGT	TTTCGCCCCG	AAGAACGTTT	TCCAATGATG	AGCACTTTTA	AAGTTCGCT	900
ATGTGGCGCG	GTATTATCCC	GTGTTGACGC	CGGGCAAGAG	CAACTCGGTC	GCCGCATACA	960
CTATTCTCAG	AATGACTTGG	TTGAGTACTC	ACCAGTCACA	GAAAAGCATC	TTACGGATGG	1020
CATGACAGTA	AGAGAATTAT	GCAGTGCTGC	CATAACCATG	AGTGATAACA	CTGCGGCCAA	1080
CTTACTTCTG	ACAACGATCG	GAGGACCGAA	GGAGCTAACC	GCTTTTTTGC	ACAACATGGG	1140
GGATCATGTA	ACTCGCCTTG	ATCGTTGGGA	ACCGGAGCTG	AATGAAGCCA	TACCAAACGA	1200
CGAGCGTGAC	ACCACGATGC	CTACAGCAAT	GGCAACAACG	TTGCGCAAAC	TATTAACCTG	1260
CGAACTACTT	ACTCTAGCTT	CCCGGCAACA	ATTAATAGAC	TGGATGGAGG	CGGATAAAGT	1320
TGCAGGACCA	CTTCTGCGCT	CGGCCCTTCC	GGCTGGCTGG	TTTATTGCTG	ATAAATCTGG	1380
AGCCGGTGAG	CGTGGGTCTC	GCGGTATCAT	TGCAGCACTG	GGGCCAGATG	GTAAGCCCTC	1440
CCGTATCGTA	GTTATCTACA	CGACGGGGAG	TCAGGCAACT	ATGGATGAAC	GAAATAGACA	1500
GATCGCTGAG	ATAGGTGCCT	CACTGATTAA	GCATTGGTAA	CTGTGAGACC	AAGTTTACTC	1560
ATATATACTT	TAGATTGATT	TAAAACCTCA	TTTTTAATTT	AAAAGGATCT	AGGTGAAGAT	1620
CCTTTTTGAT	AATCTCATGA	CCAAAATCCC	TTAACGTGAG	TTTTTCGTTC	ACTGAGCGTC	1680
AGACCCCGTA	GAAAAGATCA	AAGGATCTTC	TTGAGATCCT	TTTTTTCTGC	GCGTAATCTG	1740
CTGCTTGCAA	ACAAAAAAC	CACCGCTACC	AGCGGTGGTT	TGTTTGCCGG	ATCAAGAGCT	1800
ACCAACTCTT	TTTCCGAAGG	TAAGTGGCTT	CAGCAGAGCG	CAGATACCAA	ATACTGTCCT	1860

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TCTAGTGTAG	CCGTAGTTAG	GCCACCACTT	CAAGAACTCT	GTAGCACCGC	CTACATACCT	1920
CGCTCTGCTA	ATCCTGTTAC	CAGTGGCTGC	TGCCAGTGGC	GATAAGTCGT	GTCTTACCGG	1980
GTTGGACTCA	AGACGATAGT	TACCGGATAA	GGCGCAGCGG	TCGGGCTGAA	CGGGGGGTTT	2040
GTGCACACAG	CCCAGCTTGG	AGCGAACGAC	CTACACCGAA	CTGAGATACC	TACAGCGTGA	2100
GCTATGAGAA	AGCGCCACGC	TTCCCGAAGG	GAGAAAGGCG	GACAGGTATC	CGGTAAGCGG	2160
CAGGGTCGGA	ACAGGAGAGC	GCACGAGGGA	GCTTCCAGGG	GGAAACGCCT	GGTATCTTTA	2220
TAGTCCTGTC	GGGTTTCGCC	ACCTCTGACT	TGAGCGTCGA	TTTTTGTGAT	GCTCGTCAGG	2280
GGGGCGGAGC	CTATGGAAAA	ACGCCAGCAA	CGCGGCCTTT	TTACGGTTCC	TGGCCTTTTG	2340
CTGGCCTTTT	GCTCACATGT	TCTTTCCTGC	GTTATCCCCT	GATTCTGTGG	ATAACCGTAT	2400
TACCGCCTTT	GAGTGAGCTG	ATACCGCTCG	CCGCAGCCGA	ACGACCGAGC	GCAGCGAGTC	2460
AGTGAGCGAG	GAAGCGGAAG	AGCGCCTGAT	GCGGTATTTT	CTCCTTACGC	ATCTGTGCGG	2520
TATTTACACAC	CGCATATATG	GTGCACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	2580
AGCCAGTATA	CACTCCGCTA	TCGCTACGTG	ACTGGGTCAT	GGCTGCGCCC	CGACACCCGC	2640
CAACACCCGC	TGACGCGCCC	TGACGGGCTT	GTCTGCTCCC	GGCATCCGCT	TACAGACAAG	2700
CTGTGACCGT	CTCCGGGAGC	TGCATGTGTC	AGAGGTTTTT	ACCGTCATCA	CCGAAACGCG	2760
CGAGGCAGCA	GATCAATTCG	CGCGGAAGG	CGAAGCGGCA	TGCATTTACG	TTGACACCAT	2820
CGAATGGTGC	AAAACCTTTC	GCGGTATGGC	ATGATAGCGC	CCGGAAGAGA	GTCAATTCAG	2880
GGTGGTGAAT	GTGAAACCAG	TAACGTTATA	CGATGTCGCA	GAGTATGCCG	GTGTCTCTTA	2940
TCAGACCGTT	TCCCGCGTGG	TGAACCAGGC	CAGCCACGTT	TCTGCGAAAA	CGCGGGAAAA	3000
AGTGGAAGCG	GCGATGGCGG	AGCTGAATTA	CATTCCCAAC	CGCGTGGCAC	AACAACGGC	3060
GGGCAAACAG	TCGTTGCTGA	TTGGCGTTGC	CACCTCCAGT	CTGGCCCTGC	ACGCGCCGTC	3120
GCAAATTGTC	GCGGCGATTA	AATCTCGCGC	CGATCAACTG	GGTGCCAGCG	TGGTGGTGTG	3180
GATGGTAGAA	CGAAGCGGCG	TCGAAGCCTG	TAAAGCGGCG	GTGCACAATC	TTCTCGCGCA	3240
ACGCGTCAGT	GGGCTGATCA	TTAACTATCC	GCTGGATGAC	CAGGATGCCA	TTGCTGTGGA	3300
AGCTGCCTGC	ACTAATGTTT	CGGCGTTATT	TCTTGATGTC	TCTGACCAGA	CACCCATCAA	3360
CAGTATTATT	TTCTCCCATG	AAGACGGTAC	GCGACTGGGC	GTGGAGCATC	TGGTCGCATT	3420
GGGTCACCAG	CAAATCGCGC	TGTTAGCGGG	CCCATTAAGT	TCTGTCTCGG	CGCGTCTGCG	3480
TCTGGCTGGC	TGGCATAAAT	ATCTCACTCG	CAATCAAATT	CAGCCGATAG	CGGAACGGGA	3540
AGGCGACTGG	AGTGCCATGT	CCGGTTTTCA	ACAAACCATG	CAAATGCTGA	ATGAGGGCAT	3600
CGTTCCCACT	GCGATGCTGG	TTGCCAACGA	TCAGATGGCG	CTGGGCGCAA	TGCGCGCCAT	3660
TACCGAGTCC	GGGCTGCGCG	TTGGTGCGGA	TATCTCGGTA	GTGGGATACG	ACGATACCGA	3720
AGACAGCTCA	TGTTATATCC	CGCCGTCAAC	CACCATCAAA	CAGGATTTTC	GCCTGCTGGG	3780
GCAAACCAGC	GTGGACCGCT	TGCTGCAACT	CTCTCAGGGC	CAGGCGGTGA	AGGGCAATCA	3840
GCTGTTGCCC	GTCTCACTGG	TGAAAAGAAA	AACCACCCTG	GCGCCAATA	CGCAAACCGC	3900
CTCTCCCCGC	GCGTTGGCCG	ATTCATTAAT	GCAGCTGGCA	CGACAGGTTT	CCCGACTGGA	3960
AAGCGGGCAG	TGAGCGCAAC	GCAATTAATG	TGAGTTAGCG	CGAATTGATC	TG	4012

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2) Zip Competent Cell BL21(DE3)

The Zip Competent Cell BL21(DE3) is designed for a rapid transformation procedure. It requires neither heat shock nor culture after heat shock. Transformation of the Zip Competent Cell BL21(DE3) is completed within about 5 minutes. The time-saving procedure is a great benefit for researchers and experimenters.

The strain, BL21(DE3), is one of the popular strains for the protein expression. BL21(DE3) contains the λ DE3 lysogen, which expresses T7 RNA polymerase under the control of the *lacUV5* promoter¹⁾. Upon addition of isopropyl-1-thio- β -D-galactopyranoside (IPTG), T7 RNA polymerase is expressed and it induces a high-level protein expression from T7 promoter driven expression vectors. *E. coli* BL21(DE3) strain is a derivative of *E. coli* B strain and lacks both the *lon* protease and the *ompT* membrane protease which may degrade expressed proteins.

| Genotype of *E. coli* strain BL21(DE3) :

F⁻ *ompT hsdS*(r_B⁻ m_B⁻) *gal dcm* λ (DE3) (λ (DE3): *lacI, lacUV5-T7 gene 1, ind1, sam7, nin5*)

| Storage condition of Zip Competent Cell BL21(DE3) :

Stable at -80°C with little or no loss in transformation efficiency for 12 months from the date of receipt. Competent cells are sensitive to variation in temperature. Must be stored at -80°C. Upon receipt, store the Zip Competent Cell BL21(DE3) in a freezer at -80°C directly from the dry ice shipping box.

| Handling of competent cells :

- Competent cells are sensitive to mechanical shock. Excessive mixing should be avoided.
- After thawing competent cells on ice, cells tend to lose transformation efficiency gradually. Transformation should be started immediately after the cells have thawed on ice.
- Use of refrozen competent cells is not recommended.

| Quality Control of Zip Competent Cell BL21(DE3):

Transformation was carried out according to the method described in this Product Information using supercoiled pUC19 plasmid. Transformants were plated on LB plates containing 50 μ g/mL ampicillin. The efficiency was confirmed to be greater than 2×10^6 cfu/ μ g.

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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.

	T7 promoter		XbaI	
GATCCCGCGA	AATTAATACG	ACTCACTATA	GGGAGACCAC	AACGGTTTCC
AspProAlaL	ysLeuIleAr	gLeuThrIle	GlyArgProG	lnArgPhePr
			NdeI	6×His
AATTTTGTTT	AACTTTAAGA	AGGAGATATA	CATATGCGGG	GTTCTCATCA
AsnPheVal*	**Leu***Gl	uGlyAspIle	HisMetArgG	lySerHisHi
				EK
CATGGTATGG	CTAGCATGAC	TGGTGGACAG	CAAATGGGTC	GGGACGATGA
HisGlyMetA	laSerMetTh	rGlyGlyGln	GlnMetGlyA	rgAspAspAs
	KpnI	EcoRI	Sali	NotI
CCCCGGGTAC	CGAGCTCGAA	TTCGATTTTCG	TCGACAAGCT	TAGCGGCCGC
	SmaI	SacI	HindIII	↑
ProArgValP	roSerSerAs	nSerIleSer	SerThrSerL	euAlaAlaAl
				aVal***Ser

EK: Enterokinase recognition sequence (AspAspAspAspLys↓)

ATG: start codon

TAA: stop codon

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| Transformation:

- Materials to be supplied by user
 - LB plates containing an appropriate antibiotic
 - Ice bucket with ice
 - Sterile spreaders

- Transformation procedure

1. Thaw one tube of competent cells on ice. One tube contains 100 μ L of cells for each transformation.
2. Add DNA sample* directly into the competent cells and mix by flicking the tube.

*¹ The volume of DNA sample should not exceed 5 % of that of competent cells (i.e. for 100 μ L of competent cells, use ≤ 5 μ L).

*² If it is predicted that you will have too many colonies on the LB plate, dilute the plasmid solution with autoclaved water.

3. Leave the tube on ice for 5 minutes.
4. Spread the 100 μ L of the cells to a 37°C pre-warmed LB agar plate containing an appropriate antibiotic.
5. Incubate the plates at 37°C overnight.

| 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.

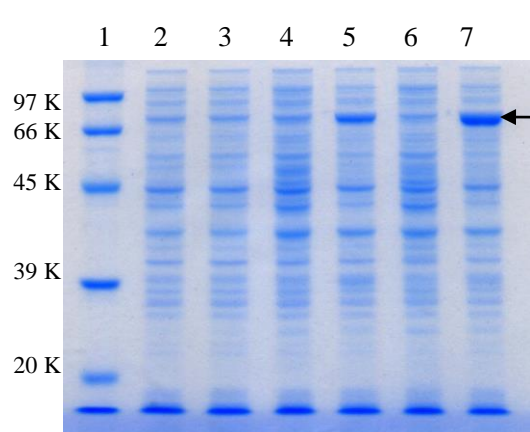
| Analysis:

1. Suspend the precipitated cells (from the 1 mL culture) in 200 μ L of 1 \times PBS buffer.
2. Mix an aliquot of the suspension (e.g., 100 μ L) with an equal volume of 2 \times 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 μ L) to SDS-PAGE. Western blot will help analyzing the expression of the target protein.

- 2 \times SDS sample buffer : 2 % sodium dodecyl sulfate, 5 % 2-mercaptoethanol, 20 % glycerol, 0.02 % BPB, 62.5 mM Tris-HCl, pH6.8

- 1 \times PBS buffer.: 20 mM sodium phosphate, 150 mM sodium chloride, pH7.4

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An arrow shows the expressed 70 KDa proteins. Only induced cells expressed 70 KDa proteins.

Figure of protein expression from pETIA

A gene of 70 KDa protein was cloned into pETIA (pETIA/70K). BL21(DE3) cell was transformed with the pETIA/70K, one of colonies were cultured overnight and transferred to two tubes (#1, #2) containing culture medium. IPTG was added to only tube #2 when the OD₆₀₀ reaches 0.5. At each stage, OD₆₀₀ of the culture was determined and the same amount of cells were lysed and subjected to 10 % polyacrylamide gel SDS electrophoresis.

Lane 1: DynaMarker Protein Eco (#DM610)

Lane 2, 3 : Cells from tubes #1 and 2 before induction.

Lane 4 : Cells (tubes #1), two hours after OD0.5.

Lane 5 : Cells (tubes #2), two hours after induction

Lane 6 : Cells (tubes #1), 4 hours after OD0.5.

Lane 7 : Cells (tubes #2), 4 hours after induction

● 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:

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

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

Reference:

- Studier, F.W. and Moffatt, B.A., *J. Mol. Biol.* 189 (1986) 113–130.
- Moffatt, B.A. and Studier, F.W., *Cell* 49 (1987) 221-227
- 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.

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Related Products:

DS250	Competent Cell BL21(DE3)	DS258	Electrocompetent Cell BL21(DE3)
DS260	Competent Cell BL21(DE3)pLysS		

Purchaser Notification:

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