Cat. # 9120 - 9025

For Research Use

TaKaRa

Chaperone Competent Cell BL21 Series

Product Manual

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Precautions for use :
Because the *araB* promoter and *araC* gene derived from of *Salmonella typhimurium* are contained in Chaperone Plasmid pG-KJE8, pGro7, pKJE7, and pTf16, please follow the guideline for experiments using recombinant DNA issued by the relevant authorities and the safety committee of your organization of your country in using this product.
NOTICE :
The intellectual property of the plasmids supplied in this product is owned by Takara Bio Inc. It is prohibited to use this product for any commercial purpose or to transfer this product to a third party. Individual license agreement must be concluded with Takara Bio Inc. when this product is used for industrial purposes.
The modified plasmid may also be subject to the ownership of Takara Bio Inc. Accordingly, to use the modified plasmid for any commmercial purpose or to transfer it to a third party requires a prior contact to Takara Bio Inc.
The use of this product is limited for research purposes. It must not be used for clinical purposes or for *in vitro* diagnosis.

I. Description

The Chaperone Competent Cell BL21 Series consists of the *Escerichia coli* strain BL21 transformed by each 5 types of plasmids included in Chaperone Plasmid Set (Cat. #3340).
It is known that molecular chaperones are involved in the protein folding process, and numerous studies have been conducted to elucidate the mechanisms of *in vivo* protein folding.
The five types of plasmids (pG-KJE8, pGro7, pKJE7, pG-Tf2, pTf16) included in Chaperone Competent Cells BL21 series are designed to enable efficient expression of multiple molecular chaperones that are known to work in cooperation as a "chaperone team" for the protein folding process. It has been reported that coexpression of a target protein with one of these chaperone teams increases recovery of proteins in soluble fraction; these proteins are often unrecoverable with conventional methods, due to the formation of inclusion bodies.
E.coli BL21 is a strain derived from B strain, which has defect of lon protease and ompT outer membrane protease. It is widely used for recombinant protein expression because it often brings a high stability in expressed protein.
When performing coexpression of target protein and chaperone team by using chaperone plasmid, it usually requires three steps: 1) Transform a host *E.coli* by chaperone plasmids, 2) Prepare competent cells by using the transformant and 3) Transform the cells by a plasmid, expressing target protein. This product, however, only requires single transformation to obtain *E.coli* that coexpresses target protein and chaperone team since it contains competent cells prepared from BL21 strain transformed by chaperone plasmids. Besides the competent cells retaining the five types of plasmids, TaKaRa Competent Cells BL21 that does not contain chaperone plasmid is also available as a control.
Each Competent cells is prepared from *E.coli* BL21 strain including one of chaperone plasmids. It is useful for protein expression with pCold DNA series. This product is not intended for protein expression system utilizing T7 promoter, such as pET systems, because the BL21 strain used as a host doesn't express T7 RNA polymerase.

Note : This product cannot be used for electroporation.

II. Components

● Chaperone Competent Cells BL21 Set (Cat. #9120)

1. Chaperone Competent Cells pG-KJE8/BL21	100 μl x 3
2. Chaperone Competent Cells pGro7/BL21	100 μl x 3
3. Chaperone Competent Cells pKJE7/BL21	100 μl x 3
4. Chaperone Competent Cells pG-Tf2/BL21	100 μl x 3
5. Chaperone Competent Cells pTf16/BL21	100 μl x 3
6. TaKaRa Competent Cells BL21	100 μl x 3
7. pUC19 DNA (0.1 ng/μl)	10 μl
8. SOC Medium*	1 ml x 18

- Chaperone Competent Cells pG-KJE8/BL21 (Cat. #9121)
- Chaperone Competent Cells pGro7/BL21 (Cat. #9122)
- Chaperone Competent Cells pKJE7/BL21 (Cat. #9123)
- Chaperone Competent Cells pG-Tf2/BL21 (Cat. #9124)
- Chaperone Competent Cells pTf16/BL21 (Cat. #9125)

Cat. #9121 - 9125

1. Competent cells — 100 μl x 10
2. pUC19 DNA (0.1 ng/μl) — 10 μl
3. SOC Medium* — 1 ml x 10

* SOC Medium

2 %	Tryptone
0.5 %	Yeast extract
10 mM	NaCl
2.5 mM	KCl
10 mM	$MgSO_4$
10 mM	$MgCl_2$
20 mM	Glucose

Table1. Chaperone genes coded on each plasmid and inducer

No.	Plasmid	Chaperone	Promoter	Resistant Marker	Expression Inducer (final conc.)
1	pG-KJE8	dnaK-dnaJ-grpE groES-groEL	*araB* *Pzt-1*	Cm	L-Arabinose (0.5 - 4 mg/ml) Tetracyclin (1 - 10 ng/ml)
2	pGro7	groES-groEL	*araB*	Cm	L-Arabinose (0.5 - 4 mg/ml)
3	pKJE7	dnaK-dnaJ-grpE	*araB*	Cm	L-Arabinose (0.5 - 4 mg/ml)
4	pG-Tf2	groES-groEL-tig	*Pzt-1*	Cm	Tetracyclin (1 - 10 ng/ml)
5	pTf16	tig	*araB*	Cm	L-Arabinose (0.5 - 4 mg/ml)

<Available expression plasmid>

The chaperone plasmids carry a pACYC origin of replication plus a chloramphenicol resistance which allows their use with most common ColE1-type plasmids having ampicillin resistance gene as a marker. As each chaperone gene is located at the downstream of *araB* or *Pzt-1* (*tet*) promoter, separate expression of chaperones and target proteins can be accomplished if the target gene is placed under the control of a different promoter, e.g. *lac*. The plasmids includes the gene (araC or tetR) required for each promoter. However, this product is not available for the combination use with a plasmid containing chloramphenicol resistance gene.

III. Storage

-80℃

Note : If it is not stored at -80℃, transformation efficiency may decrease. In this case, it is recommended to confirm the efficiency by using supplied pUC19 prior to use. Never store in liquid nitrogen.

IV. Protocol

1) Thaw Competent Cells in an ice bath just before use.
2) Gently mix cells and transfer 100 μl into a polypropylene tube (FALCON, #352059 or 352057).
   Note : Do not use a vortex to mix cells.
3) Add a plasmid for a target protein expression (≦10 ng is recommended.)
4) Keep in the ice bath for 30 min.
5) Incubate cells for 45 sec. at 42℃.
6) Stand to the ice bath for 1 - 2 min.
7) Add SOC Medium (pre-incubated at 37℃) up to a final volume of 1 ml.
8) Incubate with shaking (160 - 225 rpm) for 1 hour at 37℃.
9) Plate an appropriate volume*1 on L-broth plate*2.
10) Incubate overnight at 37℃.
   * 1 ≦ 100 μl is recommended for plating on dish with ∅ 9cm.
   * 2 Add chrolamphenicol and appropriate drug to select both of chaperone plasmid and expression plasmid for Chaperone Competent Cells. Add only drug to select expression plasmid for TaKaRa Competent Cells BL21.

Precautions

Read these precautions before use and follow them when using this product.

1) Only required vials of competent cells should be taken out of -80℃. Place the vials in a dry ice/EtOH bath immediately upon removal from -80℃. Keep cells in bath until you are ready to proceed.
2) 1.5 ml microtube is available for transformation instead of FALCON tube, which may lower the efficiency.
3) When using 100 μl of competent cell, apply high-purified plasmid DNA in less than 10 ng. If not, transformation efficiency might decrease.
4) When changing an experiment scale (e.g. the volume of competent cell) or using other tube, optimum condition should be studied.For example, the incubation should be performed at 42℃ for 60 sec in case of using 1.5 ml microtube.
5) L-broth or ψ b-broth can be used instead of SOC Medium. In this case, lower efficiency might be obtained.
6) When diluting the transformation mixture before plating, use SOC Medium.
7) L-broth plate:

Ingredient	per liter water
Tryptone	10 g
Yeast extract	5 g
NaCl	5 g

   Adjust to pH 7.5 with 1N NaOH, add agar to the concentration of 1.5 %, and autoclave.
8) It is not recommended to freeze and store the thawed competent cells. If do, freeze in a dry ice/EtOH bath and return to -80℃. The transformation efficiency will decrease by more than one magnitude.
9) The chaperone plasmids carry a replication origin of pACYC and a chloramphenicol resistance gene (Cm^r) which allows their use with common ColE1-type plasmids containing an ampicillin resistance gene. But they cannot be used with a plasmid containing Cm^r gene as a drug-resistant gene.

V. Coexpression Experiment

An media for expression of a target protein should be added with a antibiotic for a plasmid selection for a target protein, chloramphenicol (20 μg/ml) for selection of chaperone plasmid, and expression inducer for chaperone plasmid (Refer to the Table 1at page 4). When growth inhibition occurs, add the inducer for chaperone gene just before the induction of target protein expression. The species of chaperone and culture condition (e.g. media, culture temperature, airlation, timing for induction, inducer concentration, induction time) varies depending on a target protein. The optimal culture condition should be determined according to a target protein.

An example of coexpression using pCold I DNA (Cat. #3361)
cloned with a target gene and chaperone plasmid is shown below.

1. Prepare L-broth containing 20 μg/ml chloramphenicol and 50 - 100 μg/ml of ampicilin for plasmid expression, and 0.5 - 4 mg/ml L-arabinose and/or 1 - 10 ng/ml tetracycline. When using the plasmid pG-KJE8, both arabionose and tetracycline are used. For pGro7, pKJE7 and pTf16, L-arabinose alone is added. Only tetracycline is added in case of pG-Tf2.
   ＊ Please try with 0.5 mg/ml L-arabinose and 5 ng/ml tetracycline at first. Tetracycline at a low concentration would not affect the growth of *E.coli*.
2. Culture the transformant retaining pCold I DNA and Chaperone plasmid with shaking at 37℃.
3. When the OD_{600} of the culture solution reaches 0.4 - 0.6, leave at 15℃ for 30 min.
4. Add IPTG at the final concentration of 0.1 - 1.0 mM, then culture with shaking at 15℃ for 24 hours.
5. After completion of the culture, verify the expression and solubility of a target protein by SDS-PAGE or an activity assay.

VI. Transformation Efficiency

Chaperone Competent cells :
1 ng of pUC19 was transformed and selected by Cm+, Amp+ selective media plating.

TaKaRa Competent Cell BL21 :
1 ng of pUC19 was transformed and selected by Amp+ selective media plating.

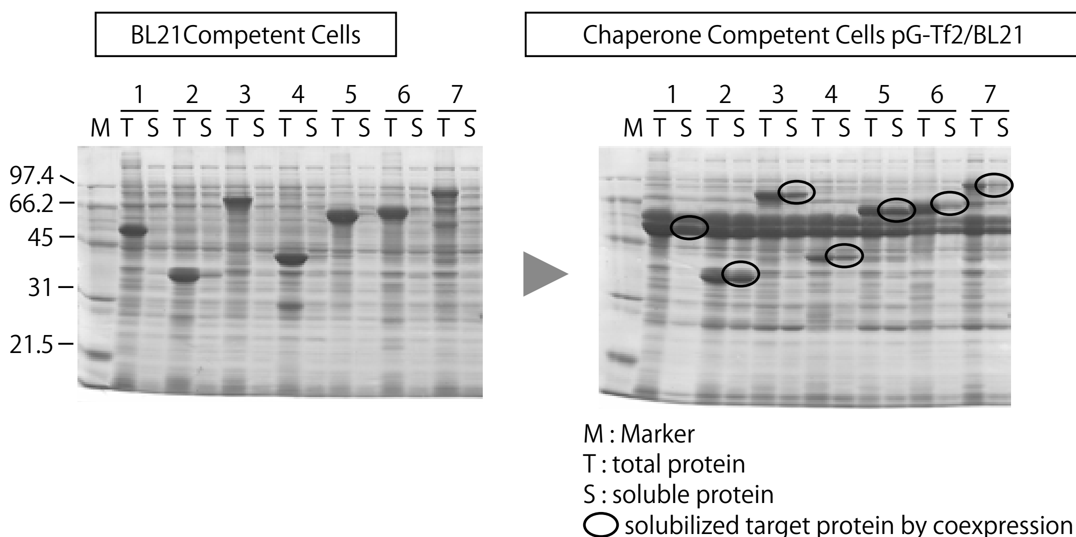
Transformation efficiency $\geqq 1 \times 10^6$ cfu/μg pUC19

VII. Genotype

E.coli BL21 : F^-, *ompT*, *hsdS*$_B$ (r_B^- m_B^-), *gal*, *dcm*

VIII. Experimental Example

Chaperone Competent Cells were used for expression of 7 human genes which were not successful in protein expression with pCold I DNA alone.
Chaperone Competent Cells pG-Tf2/BL21 was used for coexpression with chaperone plasmid.
The experiment was proceeded by following the protocol in V. Coexpression Expreiment.
The results confirmed that the target protein insoluble in the expression with pCold I DNA alone was solubilized by the coexpression with Chaperone plasmid.



M : Marker
T : total protein
S : soluble protein
○ solubilized target protein by coexpression

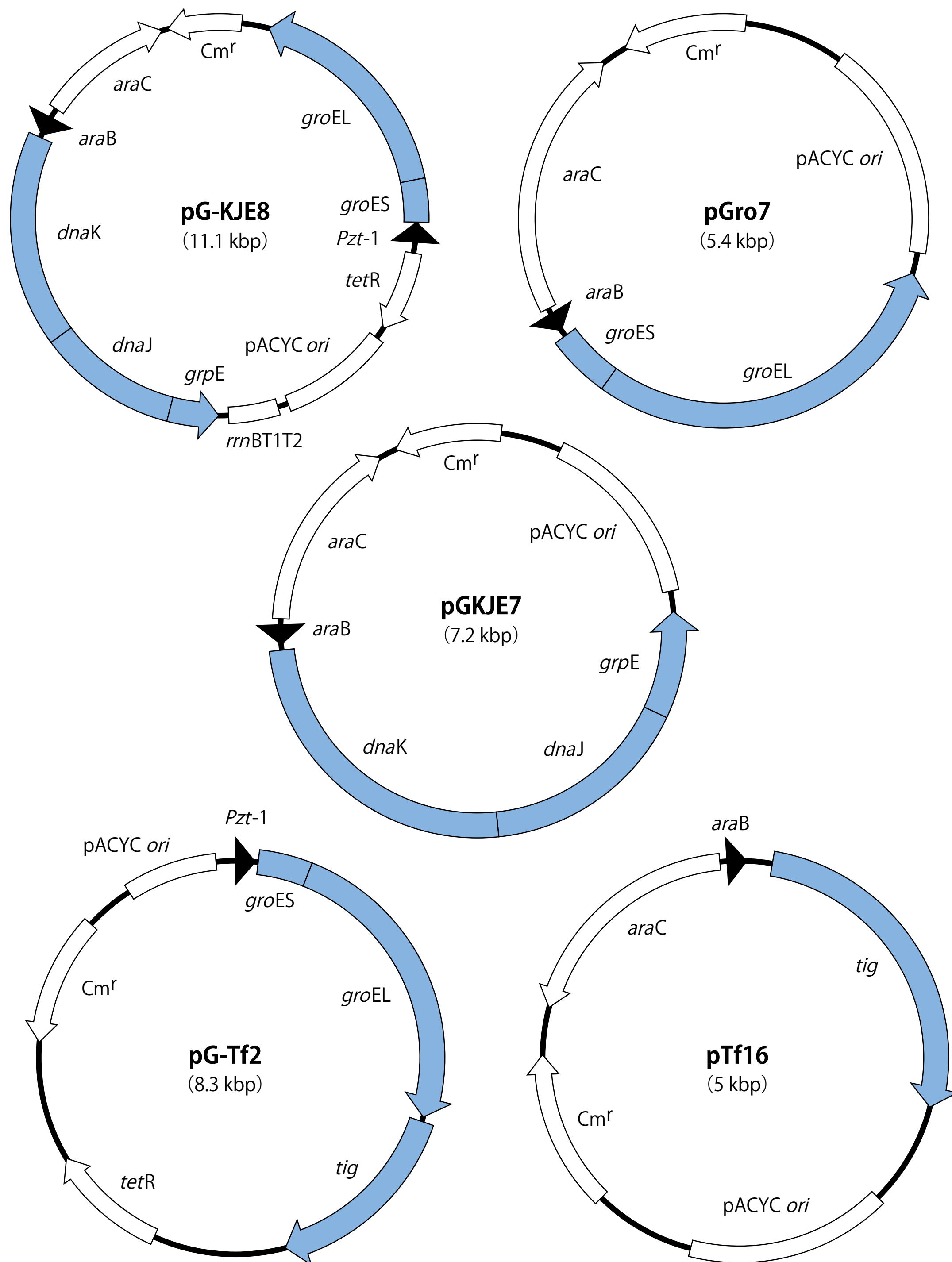
IX. Q & A

Q1 : What the size of expressed chaperone proteins are?
A1 : GroEL: 60 kDa, GroES: 10 kDa, DnaK: 70 kDa, DnaJ: 40 kDa, Tf: 56 kDa, GrpE: 22 kDa. These sizes are reported in the published literature, but they can be observed in a different size at an actual electrophoresis.
For example, the band of GrpE is verified larger than of 29 kDa.

Q2 : How to purify the expressed protein?
A2 : Affinity purification utilizing His-Tag is convenient. (pCold DNAs have His-Tag sequence.) When purifying with GST-Tag, chaperone protein might be observed in SDS-PAGE after purification, which is considered as a residue of chaperone protien on glutathion resin as a result of non-specific adsorption or co-purification with target protein.
The following procedures were reported to improve the purification.
- Separate with ion exchange resin. (*Proc Natl Acad Sci USA.* (1995) **92**: 1048)
- Separate with ATP-Agarose (*J Biol Chem.* (1984) **259**: 8820)
- Wash the resin adsorbing the protein with the buffer containing 3 mM Mg-ATP.
- Incubate the resin absorbing fusion protein in the buffer containing 10 mM Mg-ATP, 5 mg/ml casein for 20 - 30 min at room temparature.

The same trouble has not been reported in the purification with His-Tag.
Therefore His-Tag purification is recommended to purify the protein expressed by using Chaperone Competent Cells.

X. Vector Map of Chaperone Plasmid



XI. References

1) Thomas J G, *et al*. *Appl Biochem Biotech*. (1997) **66**: 197-238.
2) Nishihara K, *et al*. *Appl Environ Microbiol*. (1998) **64**: 1694-1699.
3) Nishihara K, *et al*. *Appl Environ Microbiol*. (2000) **66**: 884-889.

XII. Related Products

Chaperone Plasmid Set (Cat. #3340)
pCold Vector Set (Cat. #3360)
pCold I DNA (Cat. #3361)
pCold II DNA (Cat. #3362)
pCold III DNA (Cat. #3363)
pCold IV DNA (Cat. #3364)
pCold TF DNA (Cat. #3365)
pCold ProS2 DNA (Cat. #3371)
pCold GST DNA (Cat. #3372)
IPTG (Isopropyl- β -D-thiogalactopyranoside) (Cat. #9030)

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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