Cat. # 9126

For Research Use

TaKaRa

TaKaRa Competent Cells BL21

Product Manual

v202007Da

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I. Description

E.coli BL21 is a strain derived from B strain, which has defects 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.
It is useful for protein expression with pCold I-IV, pCold TF, pCold ProS2, and pCold GST DNA. This product is not intended for protein expression system utilizing T7 promoter, such as pET systems, because the BL21 strain doesn't express T7 RNA polymerase. In addition, it is not recommended to use this strain for construction or preparation of plasmid since it is not a *recA*-defective strain (*recA*).

Note : This product cannot be used for electroporation.

II. Components

TaKaRa Competent Cells BL21	100 μl	x 10
pUC19 DNA (0.1 ng/μl)	10 μl	
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

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 the supplied pUC19 DNA prior to use an application. Do not store in a liquid nitrogen.

IV. Protocol

(1) Thaw TaKaRa Competent Cells BL21 in an ice bath just before use.
(2) Gently mix cells and transfer 100 μl into a polypropylene tube (CORNING Cat. #352059 or 352057).
Important : Do not use a vortex to mix cells.
(3) Add DNA sample (≦10 ng is recommended. The volume should be less than 10 μl.)
(4) Keep in the ice bath for 30 min.
(5) Incubate the cells for 45 sec at 42℃.
(6) Return 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 by shaking (160 - 225 rpm) for 1 hour at 37℃.
(9) Plate the suitable volume of cells on L-broth plates containing a selection reagent＊.
(10) Incubate overnight at 37℃.
＊ Please plate on less than 100 μl when you use the φ9 cm plate.

【Read these before use】

1. Place a vial of competent cells in a dry ice/EtOH bath immediately upon removal from −80℃ freezer. Keep cells in bath until you are ready to proceed.
2. When using 100 μl of competent cell, apply high-purified plasmid DNA in less than 10 ng. If not, transformation efficiency might decrease.
3. When changing an experiment scale such as the volume of competent cells or tube type, optimum condition should be considered.
4. Use TE buffer for plasmid DNA preparation. High salt concentration in DNA solution may decrease transformation efficiency.
5. L-broth or φb-broth can be used instead of SOC Medium. In this case, lower efficiency might be obtained.

L-broth:

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

Adjust to around pH 7.5 with 1N NaOH and autoclave.

φb-broth:

Ingredient	per liter water
Tryptone	20 g
Yeast extract	5 g
$MgSO_4$, $7H_2O$	5 g

Adjust to around pH 7.5 with 1N KOH and autoclave.

6. When diluting the transformant before plating, use SOC Medium which has been added in the step (7) of Protocol.
7. It is not recommended to freeze and store the thawed competent cells.
However, if necessary, freeze in a dry ice/EtOH bath and return to −80℃.
The transformation efficiency will decrease by at least one order of magnitude.

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V. Quality

1 ng of pUC19 DNA was transformed and selected by Amp^+ selective media plating.
Transformation efficiency ≧ 1 x 10^6 colonies/μg pUC19 DNA

VI. Genotype

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

VII. Reference

Hanahan D. *J Mol Biol*. (1983) **166**: 557.

VIII. Related products

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）
Chaperone Competent Cell BL21 Series (Cat. #9120-9125)

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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