Cat. # 9057

For Research Use

TaKaRa

E.coli DH5α Competent Cells

Product Manual

v202001Da

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I. Components

E.coli DH5 α Competent Cells		100 μl x 10
pUC19 plasmid (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

II. Storage

-80℃

Note: If it is not stroed at -80℃, transformation efficiency may decrease.
In this case, it is recommended to confirm the efficiency by using supplied pUC19 prior to use an application.
Do not store in liquid nitrogen.

III. Description

E. coli DH5 α Competent Cells, prepared by Hanahan's method modified by Takara Bio, is a host for transformation. It can be used for Blue / White screening utilizing the activity of β-galactosidase (α-complementation) in combination use of pUC vectors. As this strain does not carry *lac* I^q, basically IPTG is not needed. Therefore, *E. coli* DH5 α Competent Cells allows easy selection of recombinant clones with X-Gal when constructing gene library or subcloning recombinant plasmid.

X-Gal : 5-Bromo-4-Chloro-3-Indolyl-β-D-Galactoside

III Protocol

Transformation with a plasmid vector

1) Thaw *E.coli* DH5 α Competent Cells in an ice bath just before use.
2) Gently mix cells and transfer 100 μl into a 14 ml round-bottom tube (BD Falcon, code. 352059 or 352057).
   Note: Do not use a vortex to mix cells.
3) Add DNA sample (≦ 10 ng is recommended.)
4) Keep in the ice bath for 30 min.
5) Incubte 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 an appropriate amount of culture on selective medium.*
10) Incubate overnight at 37℃.

* Plate no more than 100 μl for a φ 9 cm plate. If necessary, dilute the culture with the same medium as used in step 7).

【Please read before proceeding】

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) You may use 1.5 ml microcentrifuge tubes instead of 14 ml round bottom tubes (BD, Code. 352059 or 352057, etc.) for transformation, but it may reduce efficiency.
3) For 100 μl of competent cell, use 10 ng or less of high-purified sample DNA. If not, transformation efficiency might decrease.
4) If you change the quantity of competent cells, or type of tubes used, it might be necessary to reevaluate the conditions. For example, when using 1.5 ml microcentrifuge tubes, heat shock for 60 sec at 42℃ rather than 45 sec.
5) L-broth or φ b-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 1 N NaOH and autoclave.

φ b-broth:

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

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

6) When adding X-Gal, follow the procedures described as below:
   - Add 20 mg/ml X-Gal (dissolved in dimethylformamide) at a ratio of 200 - 300 μl/100 ml agar media.
7) DH5α can be used for the replication of M13mp vectors.
   But the strain can not form plaques, as it does not carry F factor.
8) It is not recommended to refreeze and store the thawed competent cells. However, if necessary, freeze in a dry ice/EtOH bath and return to -80℃. The transformation efficiency can be lowered by more than one magnitude.

V. Quality

1. Transformation efficiency
   1 ng of pUC19 was transformed and selected by Amp^+ selective media plating.
   Transformation efficiency > 1 x 10^8 transformants / μg pUC19

2. Stability of F' plasmid:
   When transformed with a pUC19 plasmid, blue colonies appeared on an L-agar plate containing 100 μg/ml ampicillin and 60 μg/ml X-Gal.

VI. Genotype

E.coli DH5 α :
F^-, φ 80d*lacZ*ΔM15, Δ(*lacZYA-argF*)U169, *deoR*, *recA1*, *endA1*, *hsdR17*(r_K^-, m_K^+), *phoA, supE44*, λ $^-$, *thi*-1, *gyrA96*, *relA1*

VII. Cell density

1 - 2 x 10^9 bacteria /ml

VIII. Reference

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

IX. Related products

E. coli Electro-Cells (Cat. #9027)
pUC19 DNA (Cat. #3219)
X-Gal (5-Bromo-4-Chloro-3-Indolyl-β-D-Galactoside) (Cat. #9031)