Cat. # 9027

For Research Use

TaKaRa

E. coli DH5α Electro-Cells

Product Manual

v202002Da

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I. Description

E. coli DH5α Electro-Cells are specially to be best appropriate for electroporation method. Electroporation method is used to transfer DNA into a cells by breaking the cytoplasmic membrane with high voltage pulse.
In addition, *E. coli* DH5α Electro-Cells offers high transformation efficiency and good reproducivility, it is especially useful in transferring small amount of sample into a *E.coli* in less time.
E. coli DH5α is a host for Blue/White screening utilizing the activity of β-galactosidase (α-complementation) in combination use of pUC vectors. As this strain does not carry *lacI*^{q}, basically IPTG is not needed. Therefore, The Electro-Cells allows easy selection of recombinant DNA with X-Gal when constructing gene library or subcloning recombinant plasmid.

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

II. Components

E. coli DH5α Electro-Cells	50 μl x 10
pUC19 plasmid (10 pg/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 : Store at -80℃ or lower. If the storage temperature is not maintained consistently, the transformation efficiency will be reduced. You may determine the transformation efficiency of stored cells by using the included pUC19 control.
Do not store in liquid nitrogen.

IV. Protocol

Transformation into a plasmid vector

(1) Thaw *E. coli* DH5 α Electro-Cells (50 μl) in an ice bath just before use.
(2) Add 1 - 2 μl of DNA solution＊1 into the thawed cell suspension.
(3) Transfer the mixture of cells and DNA to a cold 0.1 cm electroporation cuvette.
(4) After applying pulse＊2, immediately add 1 ml of SOC Medium (precooled in an ice bath).
(5) Incubate by shaking (160 - 250 rpm) for 1 hour at 37℃.
(6) Plate on selective media. Less than 100 μl is applied to a φ9 cm plate.
(7) Incubate overnight at 37℃.

＊1 When the sample DNA solution contains salt(s), dilute it with TE buffer or sterile purified water. Alternatively, desalination by ethanol precipitation is recommended. Ethanol precipitation should be performed when the sample DNA is prepared for use with the TaKaRa DNA Ligation Kits.

＊2 Takara Bio uses BIO-RAD MicroPulser and the electrical condition is 1.5 kV. In the case of BIO-RAD Gene Pulser, standard electrical conditions are 200 Ω, 25 μF and 1.5 kV.

【 Please read before Proceeding 】

1. Place a tube of Electro-Cells in a dry ice / EtOH bath immediately upon removal from the -80℃ freezer. Keep the cells in the bath until you are ready to proceed.
2. For 50 μl of Electro-Cells, use no more than 10 ng of high purity DNA, or the transformation efficiency may be reduced.
3. If using large size of DNA(> 7 kb), transformation efficiency might decrease.
4. If you change the quantity of Electro-Cells electroporated, it may be necessary to reevaluate the conditions.
5. L-broth or φb-broth can be used instead of SOC Medium, but efficiency may be reduced.

   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.

   φb-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 diluting, use the medium which has been added in the step (4) of IV.
7. When using X-Gal:
   Add 20 mg/ml X-Gal (dissolved in dimethylformamide) at a ratio of 200 - 300 μl/100 ml agar media.
8. DH5α can be used for the replication of M13mp vectors. But the strain can not form plaques, as it does not carry F factor.
9. Once the Electro-Cells have been thawed, refreezing for storage is not recommended. If this is unavoidable, flash freeze the cells on dry ice/ethanol and store them promptly at -80℃. However, the transformation efficiency will be lowered by at least one order of magnitude.

V. Quality

1. Transformation efficiency
   10 pg of pUC19 was transformed and selected by Amp^+ selective media plating.
   Transformation efficiency > 1 x 10^9 transformants/μg pUC19
2. α-complementation of β-galactosidase
   When pUC19 DNA was transformed, blue colonies appeared on a L-agar plate containing 100 μg/ml of ampicilin and 60 μg/ml of X-Gal.

VI. Genotype

E. coli DH5α :
F^-, Φ80d*lacZ* Δ M15, Δ (*lacZYA-argF*)U169, *deoR*, *recA1*, *endA1*, *hsdR17*(r_K^-, m_K^+), *phoA*, *supE44*, λ^-, *thi-1*, *gyrA96*, *relA1*

VII. Cell Density

> 1 x 10^{10} bacteria/ml

VIII. References

1) Dower W J, Miller J F, and Ragsdale C W. *Nucl Acids Res*. (1988)**16**: 6127.
2) Bottger E C. *Biotechniques.* (1988) **6**: 878.

IX. Related Products

E. coli DH5α Competent Cells (Cat. #9057)
pUC19 DNA (Cat. #3219)
X-Gal (5-Bromo-4-Chloro-3-Indolyl-β-D-Galactoside) (Cat. #9031)