Cat. # 9022

For Research Use

TaKaRa

E. coli JM109 Electro-Cells

Product Manual

v202101Da

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I. Description

Electro-Cells are specially prepared by Takara Bio to be best appropriate for electroporation method. Electroporation method is used to transfer DNA into a cell by breaking cytoplasmic membrane by high voltage pulse. As this electro-cells offers high transformation efficiency and good reproducibility, it is especially useful in transferring small amount of sample into a *E. coli* in less time.
As *E. coli* JM109 Electro-Cells contains F' plasmid, it can be used as a host of M13 phage vector as well as for preparation of DNA library or subcloning. When transformation of pUC vectors or transduction of M13 phage vector DNAs, recombinants can be selected easily by X-Gal and IPTG utilizing the α-complimentarity to β-galactosidase of the electro-cells.

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

II. Components

E. coli JM109 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

A. Transformation of a plasmid vector

(1) Thaw *E. coli* JM109 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 - 225 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 sample DNA solution contains salt, dilute with TE buffer or sterile purified water. Or desalting by ethanol precipitation is recommended (< 10 ng is recommended).
＊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.

B. Transduction of a M13 phage vector

(1) Follow the step (1)-(4) mentioned in IV-A.
(2) Add 200 μl of the host (*E. coli* JM109, A_{600}=0.8 - 1.0) into 3 ml of YT soft agar (preincubated at 46 - 48℃).
(3) Add a proper amount of the solution prepared at (1) into the agar, mix, and immediately spread it onto a L-plate.
(4) Incubate at room temperature for 10 - 15 min and then at 37℃ over night.

【 Please read before proceeding 】

1. Place a vial of electro-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 50 μl of electro-cells, apply high-purified sample DNA in less than 10 ng. If not, transformation efficiency might decrease.
3. If using large size of DNA(> 7 kb) transformation efficiency might decrease.
4. When changing an experiment scale, optimum condition should be considered.
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 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 SOC Medium which has been added in the step (4) of A.
7. YT soft agar :

Ingredient	per 100 ml water
Tryptone	0.8 g
Yeast extract	0.5 g
NaCl	0.5 g

   Adjust to around pH 7.6 with 1 N NaOH, add agar to the concentration of 0.6%, and autoclave.
8. Host can be prepared by culturing electro-cells.
   When DNA is inserted in a cloning site of M13 phage vector, recombinants can be easily selected by adding X-Gal and IPTG into YT-soft agar because un-recombinants appears in blue plaque.
9. YT-plate :

Ingredient	per liter water
Tryptone	8 g
Yeast extract	5 g
NaCl	5 g

   Adjust to around pH 7.5 with 1 N NaOH, add agar to the concentration of 1.5%, and autoclave.
10. When adding X-Gal or IPTG, follow the procedures described as below:
    - Add 100 mM IPTG at 100 - 300 μl/100 ml agar medium and 25 μl/3 ml soft agar.
    - Add 20 mg /ml X-Gal (dissolved in dimethylformamide) at a ratio of 200 - 300 μl/100 ml agar medium, and 50 μl/3 ml soft agar.
11. It is not recommended to freeze and store the thawed electro-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

10 pg of pUC19 was transformed and selected by Amp^+ selective media plating.
Transformation efficiency > 1 x 10^9 cfu/μg pUC19

VI. Genotype

E. coli JM109 : *recA1*, *endA1*, *gyrA96*, *thi-1*, *hsdR17*(r_K-m_K^+), e14$^-$(*mcrA*$^-$)
supE44, *relA1*, Δ (*lac-proAB*)/F'[*traD36*, *proAB*$^+$, *lacI*^{q}, *lacZ* Δ M15]

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 product

E. coli JM109 Competent Cells (Cat. #9052)