Cat. # 9021

For Research Use

TaKaRa

E. coli HB101 Electro-Cells

Product Manual

v202010Da

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I. Description

E. coli HB101 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 offer high transformation efficiency and good reproducivility, it is especially useful in transferring small amount of sample into an *E. coli* in less time.
E. coli HB101 Electro-Cells can be used for preparation of DNA library or subcloning of recombinant plasmid, that does not require blue/white selection.

II. Components

E. coli HB101 Electro-Cells	50 μl x 10
pBR322 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 pBR322 control.
Do not store in liquid nitrogen.

IV. Protocol

(1) Thaw 50 μl of *E. coli* HB101 Electro-Cells in an ice bath just before use.
(2) Add 1 - 2 μl of DNA solution* into the thawed cell suspension.
    * 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).
(3) Transfer the mixture of cells and DNA to a cold 0.1 cm electroporation cuvette.
(4) After applying pulse*, immediately add 1 ml of SOC Medium (precooled in an ice bath).
    * Takara Bio uses BIO-RAD MicroPulser and the electrical condition is 1.8 kV. In the case of BIO-RAD Gene Pulser, standard electrical conditions are 200 Ω , 25 μF, 1.8 kV.
(5) Transfer into a 14 ml round-bottom tube (CORNING Code: 352059 or 352057, etc.). Incubate by shaking (160 - 250 rpm) for 1 hour at 37℃.
(6) Plate on selective media.*
    * 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 (4).
(7) Incubate overnight at 37℃.

【 Read these before use 】

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. When changing an experiment scale, optimum condition should be considered.
4. When transferring high molecular weight DNA (> 7 kb), transformation efficiency may decrease.
5. Use TE buffer for sample DNA preparation. High salt concentration in sample DNA solution may decrease transformation efficiency.
6. 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.

L-plates :

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

Adjust to around pH 7.5 with 1N NaOH, add agar to be 1.5% and autoclave.

7. 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

10 pg of pBR322 was transformed and selected by plating to Amp^+ selective media.
Transformation efficiency : > 5 x 10^8 cfu/ μ g pBR322

VI. Genotype

E. coli HB101: *supE44*, Δ *(mcrC-mrr), recA13, ara-14, proA2, lacY1, galK2, rpsL20, xyl-5, mtl-1*, *leuB6*, *thi-1.*

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 HB101 Competent cells (Cat. #9051)
pBR 322 DNA (Cat. #3050)