Cat. # 9051

For Research Use

TaKaRa

E. coli HB101 Competent Cell

Product Manual

v201807Da

Table of Contents

I. Components

E. coli HB101 Competent Cells	100 µl x 10
pBR322 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 stored at -80℃, transformation efficiency may decrease. In this case, we recommend confirming the efficiency with the supplied pBR322 plasmid prior to using it in an application. Do not store in liquid nitrogen.

III. Description

Competent cells are prepared by Hanahan's method modified by Takara Bio and have a transformation efficiency of >1 x 10^8 cfu/µg when 100 µl of the cells are transformed by 1 ng of pBR322 plasmid.
E. coli HB101 Competent Cells can be used for preparation of a DNA library or subcloning of a recombinant plasmid.

IV. Protocols (Transformation of a Plasmid Vector)

1) Thaw *E. coli* HB101 Competent Cells in an ice bath just before use.
2) After thawing, mix the competent cells gently, and transfer 100 µl of the cells into a 14-ml, round-bottom tube (e.g., Falcon tube, etc.).
3) Add < 10 ul of plasmid DNA (≦ 10 ng is recommended).
4) Keep the tube in the ice bath for 30 min.
5) Incubate the tube for 45 sec at 42℃.
6) Return the tube 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 cell suspension on selective media.*
10) Incubate overnight at 37℃.

* Plate no more than 100 µl for a 9-cm plate.

【Precautions for use】

1) Transfer a vial of competent cells immediately to a dry ice / EtOH bath from a -80℃ freezer. Keep the cells in the bath until you are ready to proceed.
2) For transformation, you may use 1.5-ml microcentrifuge tubes instead of 14-ml, round-bottom tubes (BD Code: 352059 or 352057, etc.), but the transformation efficiency may be reduced.
3) When using 100 μl of competent cells, use less than 10 ng of highly purified plasmid DNA. Otherwise, transformation efficiency might decrease.
4) When changing an experiment's scale, optimum conditions should be considered.
5) L-broth or φ b-broth can be used instead of SOC Medium. However, transformation efficiency might be slightly decreased.

・L-broth :

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

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

6) When diluting the transformation mixture, use the medium added in the Step 7 of Section IV.
7) Once the competent cells have been thawed, refreezing for storage is not recommended. If this is unavoidable, freeze the cells quickly 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. Transformation Efficiency

1 ng of pBR322 plasmid was transformed and the transformants were selected on an Amp+ selective medium plate.
Transformation efficiency $\geqq$ 1 x 10^8 cfu / μg pBR322 plasmid

VI. Genotype

E. coli HB101:
F^-, *hsd S*20(rB-, mB-), *recA*13, *ara*-14, *proA*2, *lacY*1, *galK*2, *rpsL*20 (str), *xyl*-5, *mtl*-1, *supE*44, *leuB*6, *thi*-1.

VII. Cell Density

1 - 2 x 10^9 bacteria/ml

VIII. Reference

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