# TaKaRa *E.coli* HB101 Competent Cell

Cat.# 9051
v.0611

TaKaRa

**Contents:**

TaKaRa *E.coli* HB101 Competent Cells .....10 vials x 100 μl
pBR322 plasmid (0.1 ng/ μl) .....10 μl
SOC medium*.....10 vials x 1 ml

* · SOC medium:

| | |
|---|---|
| 2% | Bacto tryptone |
| 0.5% | Bacto yeast extract |
| 10 mM | NaCl |
| 2.5 mM | KCl |
| 20 mM | $MgSO_4$ · $MgCl_2$ (each 10 mM) |
| 20 mM | Glucose |

**Specification:**

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

**Protocols:**

Transformation into a plasmid vector

1) Thaw TaKaRa *E.coli* HB101 Competent Cells in an ice bath just before use.
2) Gently mix cells and transfer 100 μl into a polypropylene tube (Falcon 2059).
   **Important:** Do not use a vortex to mix cells.
   It is important that Falcon 2059 tubes are used for the transformation protocol, as the incubation period during the heat pulse step (step 5) is critical and has been calculated for the thickness and shape of the Falcon 2059 tubes.
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 on selective media.
10) Incubate overnight at 37℃ .

**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) Freeze the unused portion of cells in dry ice / EtOH bath and return them to the -80℃ freezer.
3) When using 100 μl of competent cell, apply high-purified sample DNA in less than 10 ng. If not, transformation efficiency might decrease.
4) When changing an experiment scale, optimum condition should be considered.
5) Use TE buffer for sample DNA preparation. High salt concentration in sample DNA solution may decrease transformation efficiency.
6) 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 |
|---|---|
| Bacto tryptone | 10 g |
| Bacto yeast extract | 5 g |
| NaCl | 5 g |

Adjust to around pH7.5 with 1N NaOH and autoclave.

· φ b-broth:

| Ingredient | per liter water |
|---|---|
| Bacto tryptone | 20 g |

Bacto yeast extract .....5 g
$MgSO_4 \cdot 7H_2O$ .....5 g
Adjust to around pH7.5 with 1N KOH and autoclave.

7) When diluting, use SOC medium which has been added in the step 7) of A.

**Transformation efficiency:** 1 ng of pBR322 was transformed and selected by $Amp^+$ selective media plating.
Transformation efficiency $\geqq 1 \times 10^8$ cfu / $\mu$ g pBR322

**Genotype:** *E.coli HB101: F', hsd S20($r_B^-$, $m_B^-$), recA13, ara-14, proA2, LacY1, galK2, rpsL20 (str), xyl-5, mtl-1, supE44, leuB6, thi-1.*

**Cell density:** $1\text{-}2 \times 10^9$ cells/ml

**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 pBR322 prior to use an application.

**Reference:**
1. Hanahan, D. (1983) *J.Mol.Biol.* **166**, 557.
2. Messing, J. (1985) *Gene* **33**, 103.