TaKaRa E. coli DH5 α Competent Cells

Contents:
TaKaRa E. coli DH5 α Competent Cells ........... 10 vials x 100 μ l
pUC19 plasmid (0.1 ng / μ l) ............................................. 1 vial x 10 μ l
SOC medium* ...................................................... 10 vials x 1 ml

* SOC medium: 2% Bacto tryptone
0.5% Bacto yeast extract
10 mM NaCl
2.5 mM KCl
10 mM MgSO₄
10 mM MgCl₂
20 mM Glucose

Specification:
TaKaRa Competent Cells are prepared by Hanahan’s method modified by TaKaRa and have a transformation efficiency of > 1 x 10⁸ transformants / μ g when 100 μ l of the cells are transformed by 1 ng pUC19.
TaKaRa E. coli DH5 α Competent Cells 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 lac⁵, basically IPTG is not needed. Therefore, TaKaRa E. coli DH5 α Competent 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

Protocols:
Transformation into a plasmid vector
1) Thaw TaKaRa E. coli DH5 α Competent Cells in an ice bath just before use.
2) Gently mix cells and transfer 100 μ l into a polypropylene tube (BD Falcon 352059 or 352057).
   Important: Do not use a vortex to mix cells.
   It is important that BD Falcon 352059 or 352057 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 BD Falcon tubes.
3) Add DNA sample (≦ 10 ng is recommended.)
4) Keep in the ice bath for 30 min.
5) Incubate cells for 45 sec. at 42°C.
6) Return to the ice bath for 1-2 min.
7) Add SOC medium (pre-incubated at 37°C) up to a final volume of 1 ml.
8) Incubate by shaking (160-225 rpm) for 1 hour at 37°C.
9) Plate on selective media. Less than 100 μ l is applied to a φ 9cm plate.
10) Incubate overnight at 37°C.

Please read before proceeding:
1) Place a vial of competent cells in a dry ice / EtOH bath immediately upon removal from -80°C freezer. Keep cells in bath until you are ready to proceed.
2) When using 100 μ l of competent cell, 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) 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.

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Transformation efficiency: 1 ng of pUC19 was transformed and selected by Amp' selective media plating. Transformation efficiency ≥ 1 x 10^5 transformants / μg pUC19

α-complementation of β-galactosidase: Blue colony appeared when pUC19 DNA was transformed, then plating the transformants on a L-agar medium containing 100 μg/ml of ampicillin and 40 μg/ml of X-Gal.

Genotype: E. coli DH5 α F-, φ 80dlacZ ΔM15, Δ(lacZYA-argF)U169, deoR, recA1, endA1, hsdR17(rK-, mK+), phoA, supE44, λ-, thi-, gyrA96, relA1

Cell density: 1 - 2 x 10^9 cells / ml

Storage: -80°C

Note: If it is not stored at -80°C, transformation efficiency may decrease. In this case, it is recommended to confirm the efficiency by using supplied pUC19 prior to use an application.


Related products:
- E. coli DH5 α Electro-Cells (TaKaRa Cat.#9027)
- pUC19 DNA (TaKaRa Cat.#3219)
- X-Gal (TaKaRa Cat.#9031)
- L-broth powder (TaKaRa Cat.#T904)