PRODUCT INFORMATION

Product Name : Electrocompetent Cell DH5α
Code No. : DS228
Size : 50 µl × 5
Competency : > 1×10^9 cfu/µg (50 µl cell, pBR322)
Supplied product : SOC medium, 1 ml × 5 *This product is for research use only*

Description :

Electrocompetent Cell DH5α is designed for high efficiency transformation by electroporation and is suitable for DNA library construction and all kinds of cloning procedures. *E coli* strain DH5α is one of the standard competent cells for molecular biology applications. The DH5α cell has mutation of φ80*lacZ*ΔM15 and lacks *laq*I^q gene, which allows blue-white color screening of transformants with X-gal (IPTG is not required).

Genotype of *E coli* strain DH5α :

*supE*44, Δ*lac*U169(φ80*lacZ*ΔM15), *hsdR*17, *recA*1, *endA*1, *gyrA*96, *thi*-1, *relA*1

Quality control :

Transformation by electroporation was carried out according to the method described in this Product Information using supercoiled pBR322 plasmid. Transformants were plated on LB plates containing 50 µg/ml ampicillin. The efficiency was confirmed to be greater than 1×10^9 cfu/µg with 50 µl of the electrocompetent cells.

Storage condition :

Stable at -80℃ with little or no loss in transformation efficiency for 12 months from the date of receipt. Upon receipt, store the Electrocompetent Cell DH5α in a freezer at -80°C directly from a dry ice shipping box and store SOC medium at room temperature or at -80°C.

Handling of competent cells :

• After thawing electrocompetent cells on ice, cells tend to lose transformation efficiency gradually. Transformation should be started immediately following thawing cells on ice.
• Electroporation is temperature-dependant. Electrocompetent cells should be maintained at 0-4°C before pulsing with electroporation apparatus.
• Use of refrozen electrocompetent cells is not recommended.

Composition of SOC medium supplied :

20 g/L	tryptone
5 g/L	yeast extract
0.5 g/L	NaCl
0.186 g/L	KCl
2.4 g/L	$MgSO_4$, anhydrous
4 g/L	glucose

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Transformation (electroporation) procedure :

● Materials to be supplied by user

- Electroporation apparatus
- Sterile electroporation cuvettes (0.1 cm gap)
- LB plates with antibiotic
- Sterilized pasteur pipettes
- Sterile 14 ml polypropylene culture tubes
- 37°C shaker
- Sterile spreaders
- 37°C incubator

If blue-white screening is required to select transformants,

- 20 mg/ml X-Gal in dimethylformamide (DMF)

● Transformation

Before start:

- Chill sterile electroporation cuvettes (0.1 cm gap) on ice.
  * Electrocompetent cells should be maintained at 0-4°C before pulsing.
- Place 14 ml sterile polypropylene culture tubes containing 950 µl of SOC medium (at room temperature) for each electroporation near the electroporation apparatus.
  * It is important to add SOC medium to electrocompetent cells immediately after pulsing.
- Set up the electroporation apparatus according to the manufacturer's instructions.

1. Thaw the electrocompetent cells on ice and add DNA solution*(1-3 µl) to the cells then flick gently.
   * DNA solution*: Highly purified DNA is recommended. DNA should be dissolved in pure water or 1×TE. For ligation mixture, see "To use ligation mixture for electroporation" below.
2. Quickly transfer the mixture of cells and DNA to a chilled electroporation cuvette, and tap the mixture to the bottom of the cuvette.
   * Electroporation should be performed immediately after mixing electrocompetent cells and DNA.
3. Place the cuvette into the electroporation apparatus, then pulse.
4. Remove the cuvette from the electroporation apparatus and immediately add the 950 µl of SOC medium to the cuvette with a sterile pasteur pipette to suspend cells.
5. Transfer the cell suspension to the sterile 14 ml polypropylene culture tube. Culture the cell at 37°C for 1 hr in a shaker.
6. Spread aliquot of the cell to an LB agar plate containing appropriate antibiotic.
   If blue-white color screening is required, spread 25 µl of 20 mg/ml X-Gal on the LB agar plates and allow the reagent to absorb 30 minutes prior to inoculating cells. As DH5α does not have *lacI*^{q}, IPTG is not required basically.
7. Incubate the plate at 37°C overnight.

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● To use ligation mixture for electroporation

DNA for electroporation should be highly purified and dissolved in pure water or low-salt buffer (e.g. 1 × TE). Ligation mixture contains salts and impurities that interfere with electroporation. High concentration of salts in the mixture of cells and DNA often causes arcing during electroporation. To use ligation mixture for electroporation, DNA should be purified by phenol/chloroform extraction following ethanol precipitation or with a spin column. The phenol/chloroform extraction following ethanol precipitation gives successive results for ligation mixture prepared with DNA Ligation Kit ver. 2 (DS110).

Reference:

Sambrook, J. and Russell, D.W. (2001) Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.