ISO 9001/14001 Certified Company

MacCell™ – DH5α 10^9

Cat. No. 15054 (For 10^9 efficiency) 1 ml

DESCRIPTION

MacCell™ – DH5α 10^9 (iNtRON, Cat. No. 15054) is an all purpose cloning strain that is ideal for the construction of gene banks or for the generation of cDNA libraries. These cells are suitable for the routine cloning purpose and cDNA library construtions. The Φ80d*lac*ZΔM15 marker provides α-complementation of the β-galactosidase gene from pUC or similar vectors and, can be used for blue/white screening of colonies on bacterial plates containing IPTG and X-gal. The cell is capable of being transformed efficiently with large size of plasmid. MacCell™ – DH5α 10^9 cells are grown and made chemically competent using an optimized modification of the procedure of Inoue method, followed by strain verification and efficiency testing. The cells are provided as frozen 100 µl aliquots. The cells are guaranteed to have a transformation efficiency of ≥ 1 x 10^9 cfu/µg (DH5α 10^9 cells) when transformed with non-saturating amounts of supercoiled pUC18 plasmid DNA.

KIT CONTENTS and STORAGE CONDITION

- Competent Cell — 100µl x 10 vials (1 ml)
- SOC Medium — 1.3 ml x 4 vials (5.2 ml)
- Control plasmid DNA (pUC18 : 20 ng/µl) — 1 vial (10 µl)
- Storage condition
  : Competent cells must be placed immediately at the bottom of a – 80 ℃ freezer directly from the dry ice shipping container.

GENOTYPE

F$^-$, φ80d **lacZ△M15**, △(lacZYA-argF)U169, **deoR**, **recA1**, **endA1**, **hsdR**17(r_K^- m_K^+), phoA, supE44, λ$^-$, thi-1, gyrA96, relA1

GENERAL HANDLING

- **Keep the cold condition :** Competent cells are very sensitive to any changes in temperature. Cells must be thawed on ice. The transformation should be started immediately after the cells are thawed.
- **Gentle treatment :** Competent cells must be treated gently. Mix cells by swirling or gently tapping the reaction tube. DO NOT mix by PIPETTING or VORTEXING
- **Pre-warming Culture Plate :** Chilled plates will decrease the transformation efficiency. It is recommended that the culture plates be pre-warmed to more than 20℃ (preferably 37℃) prior to plating
- **Addition of SOC medium to transformation mixture (for outgrowth) :** When selecting with antibiotics, an outgrowth performed in SOC medium is required for efficient transformation. In most cases, this step can be omitted when selecting with ampicillin. After the transformation mixture has incubated on ice for 5~10 min, add 4 volume of SOC (200µl of SOC to 50 µl of transformation mixture and incubate for 1 hour at 37℃ with gently shaking at 180 ~ 225 rpm . Afterwards, spread the mixture directly onto pre-warmed culture plates. Reducing agent (e.g. DTT (dithiothreitol) and 2-ME (β-mercaptoethanol) are not required in this step.

BLUE/WHITE SCREENING

- Blue/White screening can be used with a variety of vectors in conjunction with MacCell™ – DH5α 10^9 cells. To use blue/white screening for recombinants, spread the transformed cells on LB plates containing 100 µg/ml ampicillin, 80 µg/ml IPTG and 80 µg/ml X-Gal. Incubate overnight at 37 ℃.
- An alternative to prepare plates containing X-Gal and IPTG is to spread 40 µl of X-Gal Soln. (40 mg/ml) and 4 µl of IPTG Soln. (400mg/ml) of α-Complementation Solution (iNtRON, Cat. No. 15032) onto LB ampicillin plates and allow these components to absorb for 30 minutes at 37℃ prior to plating cells.

PROTOCOLS

1. Pre-heat the SOC or LB medium to 37~42 ℃.
2. Thaw the competent cells on ice.
   Note : (Optional) To determine transformation efficiency, add 1 µl (diluted to 100 pg) Control plasmid DNA to 10 µl of competent cells (refer to "Transformation efficiency" section). Mix by stir or tapping and return the tube to the ice. Then follow the procedure of step 4.
3. Add a ligated products (< 5 µl) or purified plasmid DNA (< 50 ng) directly to the competent cells. Stir gently to mix and return the tube to ice, making sure that the tube is immersed on ice except for the cap. Repeat for additional samples.
   Note : Transformation efficiencies can be increased several folds by diluting the ligated products 5-fold with TE or water prior to adding the DNA to the competent cells.
4. Incubate the tubes on ice for 30 min.
5. Heat the tubes for exactly 90 sec. in a 42℃ water bath or heat block; do not shake.
   Note : This "Heat shock" step is most easily implemented if the tubes are in a rack that leaves the bottom half of the tubes exposed. Hold the rack in the water bath so that the bottom half of the tubes are submerged for 90 sec, and then place the rack on ice.
6. Place the tubes on the ice for 5 min.
7. Add 500 µl of pre-heated SOC medium (provided) or 900 µl LB medium (not provided) (w/o antibiotics) to each tubes.
   Note : Expression in Luria Broth reduces transformation efficiency a minimum of two- to three-fold.
8. Incubate for 1 hr at 37℃ shaking incubator.
9. During incubation, dry LB agar plate (contain with appropriate selective marker; antibiotics) at the 37℃ incubator.
   Note : Selection for transformants is accomplished by plating on medium containing antibiotic(s) for the plasmid-encoded drug resistance(s).
10. Dilute the experimental reactions (< 1/20) and spread 20 - 200 µl on dry LB ager plate. (w/ antibiotics)
11. Incubate the upper LB agar plate at 37℃ overnight.

TRANSFORMATION EFFICIENCY

Transformation efficiency is defined as the number of colony forming units (cfu) produced by 1 µg of supercoiled plasmid DNA and is measured by performing a control transformation reaction using a known quantity of DNA, typically 100 pg, then calculate the number of cfu formed per microgram DNA.

Equation for Transformation Efficiency (cfu/µg)

$$\frac{\text{cfu on control plate}}{\text{pg of control DNA plated}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \text{dilution rate}$$

Example:
After adding 189 µl SOC medium to 10 µl competent cells that have been transformed with 1µl of 100 pg/ul of Control Plasmid, transfer 10 µl to 190 µl SOC medium (or appropriate media) and plate 10 µl, If 25 colonies are observed on the plate, the transformation efficiency is

$$\frac{25 \text{ cfu}}{100 \text{ pg}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \frac{200 \text{ µl of mixture}}{10 \text{ µl plated}} \times \frac{100 \text{ µl cell}}{10 \text{ µl used}} \times 20$$

$= 1 \times 10^9$ cfu/µg

TRANSFORMATION GUIDELINE AND TROUBLESHOOTING

- **Storage Conditions :** Ultracompetent cells are very sensitive to even small variations in temperature and must be stored at the bottom of a – 80°C freezer. Transferring tubes from one freezer to another may result in a loss of efficiency.
- **Use of SOC medium :** Transformation of the supplied competent cells has been optimized using SOC as the medium for outgrowth following the heat pulse. Substitution with another outgrowth medium may result in a loss of efficiency.
- **Quantity and Volume of DNA :** The greatest efficiency is obtained from the transformation of 1 µl of 0.1 ng/µl supercoiled pUC18 DNA per 50 µl of cells. When transforming a ligation mixture, add 2.5 µl of the ligation mixture per 50 µl of cells.

TECHNICAL INFORMATION

- **Heat Pulse Duration and Temperature :** Optimal transformation efficiency is observed when cells are heat-pulsed at 42°C for 90 seconds. Efficiency decreases sharply when cells are heat-pulsed for less than 45 seconds or for more than 120 seconds. Do not exceed 42°C.
- **Blue-White Color Screening :** Blue-white color screening for recombinant plasmids is available when transforming host strains that contain the lacIqZΔM15 gene on the F´ episome with a plasmid that provides α-complementation. When lacZ expression is induced by IPTG in the presence of the chromogenic substrate X-gal, colonies containing plasmids with inserts will remain white, while colonies containing plasmids without inserts will be blue. When performing blue-white color screening, incubate the LB agar plates containing IPTG and X-gal at 37°C for at least 17 hours to allow color development. The blue color can be enhanced by subsequent incubation of the plates for two hours at 4°C. If an insert is suspected to be toxic, plate the cells on media without X-gal and IPTG. Color screening will be eliminated, but lower levels of the potentially toxic protein will be expressed in the absence of IPTG.

COMPOSITION OF BUFFERS AND SOLUTIONS

- **2 M Glucose (Not provided)**
  1. Weigh out 180.16 g of glucose powder.
  2. Dissolve in 300ml of distilled water.
  3. Transfer to a graduated cylinder and adjust volume to 500 ml with distilled water.
  4. Filter sterilize through a 0.2 μm filter.

- **IPTG Stock Solution (400 mg/ml) (Not provided)**
  1. Weigh out 2 g of IPTG powder.
  2. Dissolve in 5 ml of distilled water.
  3. Filter sterilize through a 0.2 μm filter.
  4. Store at 4 ℃.

- **LB agar medium with ampicillin (Not provided)**
  1. Weigh out 10 g Tryptone, 5 g yeast extract, 5 g NaCl and 15 g agar.
  2. Dissolve in 700 ml of distilled water.
  3. Adjust volume to 1 L with distilled water.
  4. Autoclave to sterilize and cool down the autoclaved medium to 55 ℃.
  5. Add amplicillin (final concentration 100 μg/ml).

- **X-Gal (40 mg/ml) (Not provided)**
  1. Weigh out 400 mg of X-Gal powder.
  2. Dissolve in 10 ml of dimethylforamide (DMF).
  3. Wrap the tube containing the solution in aluminum foil to prevent damage by light and store at -20°C.

- **2M Mg^{2+} Stock Solution (Not provided)**
  1. Weigh out 101.5 g of $MgCl_2 \cdot 6H_2O$ and 123.3 g of $MgSO_4 \cdot 7H_2O$.
  2. Dissolve in 300ml of distilled water.
  3. Transfer to a graduated cylinder and adjust volume to 500 ml with distilled water.
  4. Filter sterilize through a 0.2 μm filter.

  Note : Filter should be pre-rinsed with distilled water before use to remove any toxic material.

- **SOC Medium**
  1. Weigh out 2.0 g of Tryptone and 0.5 g of Yeast extract.
  2. Dissolve in 70 ml of distilled water .
  3. Add 1 ml of 1 M NaCl and 0.25 ml of 1 M KCl.
  4. Transfer to a graduated cylinder and adjust volume to 100 ml with distilled water.
  5. Autoclave to sterilize and cool down the autoclaved medium to room temperature.
  6. Add 1 ml of 2 M Mg^{2+} Stock solution and 1 ml of 2 M Glucose. (final concentration 20 mM).

QUALITY CONTROL TESTING

- MacCell™ – DH5α 10^9 cells consistently have yield more than 1 x 10^9 transformant / μg pUC18 with non-saturating amounts (100 pg) of DNA. Saturating amounts of pUC18 (above 50 ng) generate more than 1 x 10^{7-8} of ampicillin-resistant colonies in a 50 μl reaction.

RELATIVE PRODUCTS

Product Name	Cat. No.
pLUG® TA-Cloning Vector Kit	11041
pLUG® -Multi TA-Cloning Vector Kit	11051
LINKeed®Rapid DNA Ligation Kit (Version 2.0)	15023
Muta-Direct™ Site Directed Mutagensis Kit	15071
DNA-spin™ Plasmid DNA Extraction Kit	17096/17097/17098
α-Complementation Solution	15032
T4 DNA Ligase	27012 / 27013