ABLE® K Electroporation-Competent Cells

Catalog #200162

MATERIALS PROVIDED

Materials provided	Quantity	Efficiency (cfu/μg of pUC18 DNA)
ABLE® K electroporation-competent cells (green tubes)	5 × 100 µl	≥5 × 10^9
pUC18 control plasmid (0.1 ng/µl in TE buffer)	10 µl	—

Storage: Transfer the cells directly from the dry ice shipping container to the bottom of a –80°C freezer. Do not store cells in liquid nitrogen.

QUALITY CONTROL TESTING

Transformations are performed both with and without plasmid DNA using 40-µl aliquots of cells and 10 pg of pUC18 control plasmid following the protocol outlined below. Following transformation, 5-µl samples of the culture are plated in duplicate on LB agar plates with 100 µg/ml of ampicillin. The plates are incubated at 37°C overnight and the efficiency is calculated based on the average number of colonies per plate.

BACKGROUND

Stratagene has developed a method* to produce electroporation-competent cells that have the highest transformation efficiencies. These electroporation-ready cells need only be thawed, mixed with DNA, and electroporated. The ABLE® C and ABLE K strains are designed to enhance the probability of retrieving clones that are toxic to *E. coli* through the reduction of plasmid copy number by ~4- and ~10-fold, respectively, compared to the XL1-Blue strain. This copy number reduction, which is exerted on any ColE1-derived vector, decreases the levels of cloned gene products and enhances the probability that a toxic clone will be propagated.

ABLE K Genotype: *E. coli* C *lac*(LacZω$^-$) [Kan^r McrA$^-$ McrCB$^-$ McrF$^-$ Mrr$^-$ HsdR (r_K^- m_K^-)] [F′ *proAB lacI*^{q}Z*ΔM15* Tn*10* (Tet^r)]. (Genes listed signify mutant alleles. Genes on the F' episome, however, are wild-type unless indicated otherwise.)

ABLE K cells are kanamycin and tetracycline resistant. The ABLE K strain is a restriction minus (McrA$^-$, McrCB$^-$ McrF$^-$, Mrr$^-$, HsdR$^-$) derivative of *E. coli* C. The ABLE K strain contains the *lacI*^{q}Z*ΔM15* gene on the F′ episome, allowing blue-white screening for recombinant plasmids; however, since blue-white screening includes gene induction by IPTG, which increases the levels of potentially toxic proteins, blue-white color screening is not recommended for most cloning applications using the ABLE K strain.

ELECTROPORATION PROTOCOL

1. Pre-chill two sterile electroporation cuvettes (0.1-cm gap) and two sterile 1.5-ml microcentrifuge tubes thoroughly on ice. Preheat sterile SOC medium§ to 37°C.
2. Set the electroporator to a voltage setting of 1700 V (17 kV/cm field strength). If using a Bio-Rad electroporator, set the resistance at 200 Ω and the capacitance at 25 µF. For best results, chill the electroporator or perform the electroporation in a cold room.
3. Thaw the electroporation-competent cells on ice. After mixing the cells gently, aliquot 40 µl of cells into each of the two pre-chilled tubes (one tube for the experimental transformation and one tube for the pUC18 control transformation). Keep the tubes on ice.
4. Add the DNA to the cells with gentle mixing. For optimal efficiency in the experimental transformation, add 1 µl of plasmid DNA (10 pg/µl, in a low ionic strength buffer or dH_2O) to 40 µl of cells. The DNA volume may be increased up to 4 µl, but the efficiency may be reduced. Dilute the pUC18 control DNA 1:10 with sterile dH_2O, then add 1 µl of the diluted pUC18 DNA to the other 40 µl of cells.
5. Transfer the cell-DNA mixture to a **chilled** electroporation cuvette, tapping the cuvette until the mixture settles evenly to the bottom.
6. Slide the cuvette into the electroporation chamber until the cuvette sits flush against the electrical contacts.
7. Pulse the sample once, then quickly remove the cuvette. **Immediately** add 960 µl of SOC medium (held at 37°C) to resuspend the cells.
8. Transfer the cells to a sterile 14-ml BD Falcon polypropylene round-bottom tube (BD Biosciences Catalog #352059). Incubate the tube at 37°C for 1 hour with shaking at 225–250 rpm.
9. Plate 5–100 µl of the transformation mixture on LB agar plates containing the appropriate antibiotic (and containing IPTG and X-gal if color screening is desired).§ For the pUC18 control transformation, plate 5 µl of the transformation on LB-ampicillin agar plates.§

   Note *If plating <100 µl of cells, pipet the cells into a 200 µl pool of SOC medium and then spread the mixture with a sterile spreader. If plating ≥100 µl, the cells can be spread on the plates directly. Tilt and tap the spreader to remove the last drop of cells.*

10. Incubate the plates at 37°C overnight (less than 24 hours, and at least 17 hours for blue-white color screening). If blue-white screening was performed, colonies containing plasmids with inserts will remain white, while colonies containing plasmids without inserts will be blue. The blue color can be enhanced by incubating the plates for two hours at 4°C following the overnight incubation at 37°C.
11. For the pUC18 control, expect 250 colonies (≥5 × 10^9 cfu/µg pUC18 DNA). For the experimental DNA, the number of colonies will vary according to the size and form of the transforming DNA, with larger and non-supercoiled DNA producing fewer colonies.

*U.S. Patent Nos. 6,338,965 and 6,040,184.

§See *Preparation of Media and Reagents*.

Transformation Guidelines and Troubleshooting

Aliquoting Cells: Keep the cells on ice at all times during aliquoting. It is essential that the microcentrifuge tubes that the cells will be aliquoted into are placed on ice before the cells are thawed and that the cells are aliquoted directly into the pre-chilled tubes.

Cuvette Gap Width: Use a cuvette with a 0.1-cm gap to maximize the transformation efficiency and to minimize the possibility of arcing. A cuvette with a 0.2-cm gap is not recommended because the transformation efficiency is lower and the possibility of arcing is higher.

Quantity and Volume of DNA: The greatest efficiency is obtained from the transformation of 1 µl of 0.01 ng/µl of DNA per 40 µl of cells. The volume of DNA may be increased to up to 4 µl but the transformation efficiency may be reduced and the possibility of arcing may be increased if the DNA solution contains salts. A greater number of colonies may be obtained by increasing the amount of DNA added to the cells, although the overall efficiency may be lower.

Ionic Strength of DNA Solution: The sample DNA to be transformed by electroporation must be in a low-ionic-strength buffer, such as TE buffer or water. DNA samples containing too much salt will cause arcing at high voltage, possibly damaging both the sample and the machine.

Blue-White Color Screening: Blue-white color screening for recombinant plasmids is available when transforming host strains that contain the *lacI*^{q}*ZΔM15* gene on the F´ episome with a plasmid that provides α-complementation (e.g. Stratagene's pBluescript® II). 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.

Reduction of Satellite Colonies: Due to the high concentration of bacteria in electroporation, the transformation plates should be incubated at 37°C for less than 24 hours. If the incubation is to be extended beyond 24 hours, Stratagene recommends selecting for transformants on LB plates containing tetracycline (30 µg/ml) in addition to the antibiotic used to select for the transforming plasmid. Including tetracycline helps suppress satellite colony growth and does not affect the transformation efficiency. In addition, all colonies that grow in the presence of tetracycline contain an F´ episome, improving blue-white color selection. This measure for reducing growth of satellite colonies may be used for all of Stratagene's electroporation competent cells, **except** the TG1 electroporation-competent cells, which are Tet^{s}.

Preparation of Media and Reagents

LB Agar (per Liter) 10 g of NaCl 10 g of tryptone 5 g of yeast extract 20 g of agar Add deionized H_2O to a final volume of 1 liter Adjust pH to 7.0 with 5 N NaOH Autoclave	**SOB Medium (per Liter)** 20.0 g of tryptone 5.0 g of yeast extract 0.5 g of NaCl Add deionized H_2O to a final volume of 1 liter Autoclave Add 10 ml of filter-sterilized 1 M $MgCl_2$ and 10 ml of filter-sterilized 1 M $MgSO_4$ prior to use
LB–Ampicillin–Tetracycline Agar (per Liter) (Use for reduced satellite colony formation) 1 liter of LB agar, autoclaved Cool to 55°C Add 10 ml of 10 mg/ml filter-sterilized ampicillin Add 30 mg of filter-sterilized tetracycline Pour into petri dishes (~25 ml/100-mm plate)	**SOC Medium (per 100 ml)** ***Note*** *This medium should be prepared immediately before use.* 2 ml of filter-sterilized 20% (w/v) glucose or 1 ml of filter-sterilized 2 M glucose SOB medium (autoclaved) to a final volume of 100 ml
LB–Ampicillin Agar (per Liter) 1 liter of LB agar, autoclaved Cool to 55°C Add 10 ml of 10 mg/ml filter-sterilized ampicillin Pour into petri dishes (~25 ml/100-mm plate)	**TE Buffer** 10 mM Tris-HCl (pH 7.5) 1 mM EDTA

Preparation of Agar Plates for Blue-White Color Screening

To prepare plates for blue-white screening, prepare LB agar as indicated above. When adding the antibiotic, also add 5-bromo-4-chloro-3-inodlyl-β-D-galactopyranoside (X-gal) to a final concentration of 80 µg/ml [prepared in dimethylformamide (DMF)] and isopropyl-1-thio-β-D-galactopyranoside (IPTG) to a final concentration of 20 mM (prepared in sterile dH_2O). Alternatively, 100 µl of 10 mM IPTG and 100 µl of 2% X-gal may be spread on solidified LB agar plates 30 minutes prior to plating the transformations. (For consistent color development across the plate, pipet the X-gal and the IPTG into a 100-µl pool of SOC medium and then spread the mixture across the plate. Do not mix the IPTG and the X-gal before pipetting them into the pool of SOC medium because these chemicals may precipitate.)

Limited Product Warranty

Endnotes

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