ABLE® K Competent Cells

Catalog #200172

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MATERIALS PROVIDED

Materials provided	Quantity	Efficiency (cfu/μg of pUC18 DNA)
ABLE® K competent cells (yellow tubes)	5 × 0.2 ml	5×10^6–2×10^7
pUC18 control plasmid (0.1 ng/μl in TE buffer)	10 μl	—
β-Mercaptoethanol (1.42 M)	25 μl	—

Storage: Competent cells must be placed immediately at the bottom of a –80°C freezer directly from the dry ice shipping container. Do not store the cells in liquid nitrogen.

QUALITY CONTROL TESTING

Transformations are performed both with and without plasmid DNA using 100-μl aliquots of cells and 100 pg of pUC18 control plasmid following the protocol outlined below. Following transformation, 50-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

The ABLE® C and ABLE K strains are designed to enhance the probability of retrieving clones that are toxic to *E. coli* through the modulation of plasmid copy number. The ABLE C strain and the ABLE K strain reduce the copy number of common cloning vectors (any ColE1-derived vector) by ~4- and ~10-fold, respectively, compared to the XL1-Blue strain. This copy number reduction decreases the levels of cloned gene products and enhances the probability that a toxic clone will be propagated. Positive clones observed on initial screening of lambda libraries can be excised or recloned into any convenient vector and can be introduced into the ABLE strains.

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.

TRANSFORMATION PROTOCOL

1. Pre-chill two 14-ml BD Falcon polypropylene round-bottom tubes on ice. (One tube is for the experimental transformation and one tube is for the pUC18 control.) Preheat SOC medium[§] to 42°C.
2. Thaw the competent cells on ice. When thawed, gently mix and aliquot 100 μl of cells into each of the two pre-chilled tubes.
3. Add 1.7 μl of β-mercaptoethanol provided with this kit to each aliquot of cells.
4. Swirl the contents of the tubes gently. Incubate the cells on ice for 10 minutes, swirling gently every 2 minutes.
5. Add 0.1–50 ng of the experimental DNA (see *Quantity and Volume of DNA*, reverse page, for guidelines) to one aliquot of cells and add 1 μl of the pUC18 control DNA to the other aliquot. Swirl the tubes gently.
6. Incubate the tubes on ice for 30 minutes.
7. Heat-pulse the tubes in a 42°C water bath for 45 seconds. The duration of the heat pulse is **critical** for maximum efficiency.
8. Incubate the tubes on ice for 2 minutes.
9. Add 0.9 ml of preheated SOC medium and incubate the tubes at 37°C for 1 hour with shaking at 225–250 rpm.
10. Plate ≤200 μl of the transformation mixture on LB agar plates containing the appropriate antibiotic. For the pUC18 control transformation, plate 50 μl of the transformation on LB–ampicillin agar plates.[§]

    Notes *Cells may be concentrated by centrifuging at 1000 rpm for 10 minutes. Resuspend the pellet in 200 μl of SOC medium.*

    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.
11. Incubate the plates at 37°C overnight.
12. For the pUC18 control, expect 25–200 colonies (5×10^6–2×10^7 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.

[§]See *Preparation of Media and Reagents*.

Transformation Guidelines and Troubleshooting

Storage Conditions: Competent and supercompetent 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 14-ml BD Falcon polypropylene round-bottom tubes: It is important that 14-ml BD Falcon polypropylene round-bottom tubes (BD Biosciences Catalog #352059) are used for the transformation protocol, since other tubes may be degraded by β-mercaptoethanol. In addition, the duration of the heat-pulse is critical and has been optimized for these tubes.

Aliquoting Cells: Keep the cells on ice at all times during aliquoting. It is essential that the polypropylene tubes are placed on ice before the cells are thawed and that the cells are aliquoted directly into pre-chilled tubes. It is also important to use the volume of cells indicated in step 2 of the *Transformation Protocol*. Decreasing the volume will result in lower efficiencies.

Use of β-Mercaptoethanol (β-ME): β-ME has been shown to increase transformation efficiency. The β-ME provided is diluted and ready to use. A fresh 1:10 dilution (from a 14.2 M stock) may be used; however, Stratagene cannot guarantee results with β-ME from other sources.

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 reaction. A greater number of colonies may be obtained by transforming up to 50 ng DNA, although the resulting efficiency (cfu/µg DNA) may be lower. The volume of the DNA solution added to the reaction may be increased to up to 10% of the reaction volume, but the transformation efficiency may be reduced.

Heat Pulse Duration: Optimal transformation efficiency is observed when cells are heat-pulsed at 42°C for 45–50 seconds. Efficiency decreases sharply when cells are heat-pulsed for <45 seconds or for >60 seconds.

Blue-White Color Screening: Blue-white color screening for recombinant plasmids is available when transforming host strains that contain the *lacI*^{q}ZΔ*M15* gene on the F´ episome with a plasmid that provides α-complementation (e.g. Stratagene's pBluescript® II vector). 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.

Preparation of Media and Reagents

SOB Medium (per Liter)	SOC Medium (per 100 ml)
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	***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 Agar (per Liter)	**LB–Ampicillin 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	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)

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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