RapidTrans™

96-Tube Chemically Competent *E. coli*

(version C2)

Catalog Nos. 11096, 11596

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Introduction

RapidTrans™ is a 96-tube tray of competent *E. coli* giving the flexibility of using 1 to 96 tubes at your convenience. This eliminates the waste associated with freeze/thawing and aliquoting the competent cells. Each tube contains 50 µl of competent *E. coli*, which is sufficient for one reaction.

Kit Contents

Component	Quantity	Store at
Chemically competent *E. coli*	96 rxns (50 µl per tube)	–80°C
pUC19 plasmid DNA	100 ng (10 ng/µl)	–20°C
SOC medium	30 ml	Room Temperature

Important:
- To maintain high competency level, please store cells at –80°C.
- For strain genotypes and media recipes please see Appendix.

Additional Materials Required

- Ice bucket with crushed ice
- 37°C incubators (shaking and non-shaking)
- 42°C water bath
- LB agar plates containing appropriate additives (antibiotics, IPTG, X-gal, etc.)

Protocol

Preparation

1. Ensure water bath is at 42°C.
2. Pre-warm LB agar plates to 37°C.
3. The box that holds the 96 transformation tubes can be used as a rack for all stages of the procedure. For heat-shock at 42°C, remove the bottom panel prior to immersion in the water bath.

General Transformation Procedure

1. Remove required number of transformation reaction tubes from –80°C storage and place on ice to thaw.

   Note: Individual tubes can be removed by cutting cap strips with a sharp blade.

2. Add 1-5 µl of plasmid DNA/ligation to thawed cells. Mix by tapping tubes gently and replace on ice immediately.
   Do not mix by vortexing or pipetting.
   Do not add more than 5 µl (10% of competent cell volume) to reaction.
3. Incubate transformation reactions on ice for 30 minutes.
4. Heat-shock the tubes by immersing in a 42°C water bath for exactly 30 seconds.

   Note: The bottom of the 96-tube storage rack can be removed to allow for easier immersion of the tubes into the water bath.

5. Replace transformation reactions on ice for 2 minutes.
6. Aseptically add 250 µl SOC medium to each reaction.

   Note: A special reservoir is supplied with the kit to facilitate ease of addition of SOC medium when carrying out multiple transformations. Aseptically transfer SOC medium into the sterile reservoir and use a multi-channel pipette to add medium to transformation reactions.

7. Incubate tubes at 37°C for 1 hour with shaking at 225-250 rpm.
8. Using a sterile spreader, plate out 20-200 µl of each transformation on pre-warmed LB agar plates.
9. Allow plates to completely absorb any excess media.
10. Incubate inverted plates overnight at 37°C.

Control Transformation

It is recommended that you test the transformation efficiency of the cells supplied in this kit. An aliquot of pUC19 supercoiled plasmid DNA is included with the kit for this purpose. The control procedure is similar to the general transformation procedure described above, with the following additional steps.

1. Prepare a 10 pg/µl solution of pUC19 in sterile H_2O from the supplied 10 ng/µl stock solution by serial dilution. Add 1 µl of the 10ng/µl stock to 99 µl of sterile H_2O to make a 100 pg/µl solution.
2. Add 1 µl of the 100 pg/µl solution to 9 µl of sterile H_2O to make a 10 pg/µl solution. Store on ice until ready to use.
3. Transform 1 µl of the 10 pg/µl solution according to the general transformation procedure described above.
4. Plate 10 & 100 µl of the transformation reaction on two separate LB Amp plates (for easier plating add 90 µl SOC to the 10 µl transformation reaction).
5. Incubate overnight at 37°C.

Calculation of Efficiency

$$\frac{\text{No. of colonies}}{\text{10 pg transformed pUC19}} \times \frac{10^6 \text{pg}}{\text{µg}} \times \frac{\text{total transformation vol (300 µl)}}{\text{transformation vol plated}} = \frac{\text{No. of transformants}}{\text{µg pUC19}}$$

Appendix

Section 1: Strain Information

For use in cloning, plasmid preparation and library construction

TAM1: *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*Δ139 (*ara-leu*)7697 *gal*U *gal*K *rps*L *end*A1 *nup*G

1. The *mcr*A, *mcr*BC and *mrr* mutations prevent cleavage of methylated DNA. The McrA and McrBC restriction systems[1-4] cleave DNA at methylated cytosines contained in the target sequences 5´-CG-3´ for McrA and 5´-PuC- 3´ for McrBC. DNA from some sources (including human DNA) may be methylated at these sites and will therefore be cloned inefficiently in strains expressing McrA and/or McrBC. The Mrr restriction system[1, 3, 5] cleaves DNA at methylated adenines, although the precise recognition sequence is not known. DNA from some sources may be methylated at Mrr recognition sites and will therefore be cloned inefficiently in a mrr+ strain.
2. The EcoK system (*hsd*M DNA methylase, *hsd*R endonuclease and *hsd*S specificity determinant) recognizes the sequence 5´-AACNNNNNNGTT-3´[1, 6, 7]. The modification component protects the host DNA by methylation of the second A in each strand of the target sequence. DNA cloned in a *hsd*M host will be restricted if subsequently transferred into a *hsd*R+ host.
3. The *lac*ZΔM15 deletion removes the amino-terminal α peptide (amino acids 11-41) of β-galactosidase. Cloning vectors that employ Lac selection carry a gene that codes for the α peptide and rescues the *lac*ZΔM15 mutation by α complementation[8].
4. RecA is responsible for general recombination, DNA repair and phage λ induction. Mutation in *rec*A helps stabilize sequences with direct repeats.
5. EndA is a DNA-specific endonuclease I. Mutation in *end*A results in improved quality of plasmid miniprep DNA.

Section 2: Media Information

LB Agar (per liter)
10 g NaCl
10 g tryptone
5 g yeast extract
Dissolve in 950 ml of deionized H_2O
Adjust pH to 7.0 with 5 N NaOH
15 g agar
Add deionized H_2O to 1 L final volume
Autoclave and let cool to ~55°C before pouring or addition of antibiotics, etc.

SOB Medium (per liter)
20 g tryptone
5 g yeast extract
0.5 g NaCl
Dissolve in 950 ml of deionized H_2O
Add 10 ml of 250 mM KCl stock solution (1.86 g KCl/100 ml deionized H_2O)
Adjust pH to 7.0 with 5 N NaOH
Add deionized H_2O to 1 L final volume
Autoclave and let cool to ~55°C
Aseptically add 10 ml of sterile 1 M $MgCl_2$.

SOC Medium (per liter)
Aseptically add 7.2 ml of sterile 50% glucose to SOB medium.

Blue/White Screening

X-gal Stock Solution

Make a 40 mg/ml solution of X-gal in dimethylformamide (DMF). Use a glass or polypropylene tube and wrap in aluminum foil to prevent damage by light. Store at –20°C.

Plate 40 µl of X-gal stock solution on top of the LB agar and let stand for approximately 1 hour. Alternatively, add the X-gal to the molten LB agar prior to pouring plates.

Section 3: References

1. Raleigh, E.A., Leach, K. and Brent, R. (1989) in Current Protocols in Molecular Biology (F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith and K. Struhl, eds). John Wiley, New York, p. 1.4.6-1.4.10.
2. Raleigh, E.A. (1992) *Mol. Microbiol.*, 6, 1079.
3. Kelleher, J. and Raleigh, E.A. (1991) *J. Bacteriol.*, 173, 5220.
4. Sutherland, E., Coe, L. and Raleigh, E.A. (1992) *J. Mol. Biol.*, 225, 327.
5. Waite-Rees, P.A., Keating, C.J., Moran, L.S., Stalko, B.E., Hornstra, L.J. amd Benner, J.S. (1991) *J. Bacteriol.*, 173, 5207.
6. Raleigh, E.A. (1987) *Meth. Enzymol.*, 152, 130.
7. Bickle, T. (1993) in *Nucleases* (S.M. Linn, R.S. Lloyd and R.J. Roberts, eds). Cold Spring Harbor Laboratory Press, New York, p. 89-109.
8. Ullman, A. and Perrin, D. (1970) in *The Lactose Operon* (J.R. Beckwith and D. Zipser, eds). Cold Spring Harbour Laboratory Press, New York, p. 143.

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