E. coli Competent Cells: Multiple-Use Protocol

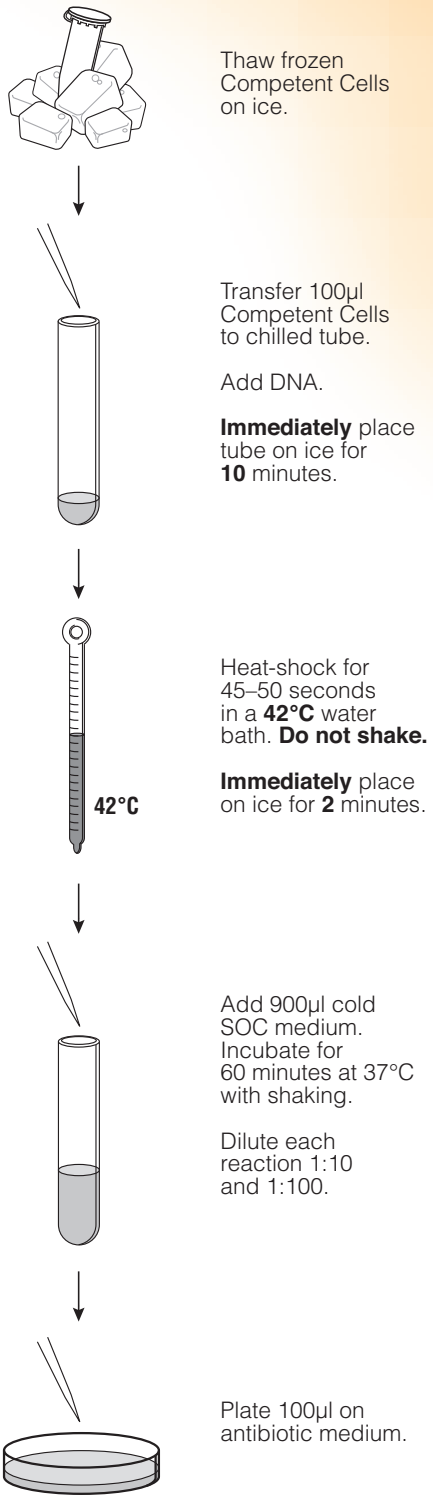
INSTRUCTIONS FOR USE OF PRODUCTS L1001, L1191, L2001 AND L2011.

Quick PROTOCOL

Standard Transformation Protocol for Multiple-Use Cells

1. Chill sterile 17 × 100mm polypropylene culture tubes on ice.
2. Thaw frozen Competent Cells on ice until just thawed.
3. Gently mix the thawed Competent Cells by flicking the tube. Transfer 100µl to each of the chilled culture tubes.
4. Add 1–50ng of DNA or 1µl (0.1ng) of Competent Cells Control DNA per 100µl of Competent Cells. Quickly flick the tube several times. (Use the Competent Cells Control DNA to determine transformation efficiency.)
5. Immediately return the tubes to ice for 10 minutes.
6. Heat-shock the cells for 45–50 seconds in a water bath at exactly 42°C. **Do not shake.**
7. Immediately place the tubes on ice for 2 minutes.
8. Add 900µl of cold (4°C) SOC medium to each transformation reaction. Incubate for 60 minutes at 37°C with shaking.
9. For each transformation reaction, dilute the cells 1:10 and 1:100. Plate 100µl of the undiluted, 1:10 and 1:100 dilutions onto agar plates containing antibiotics. Incubate the plates at 37°C for 12–14 hours or overnight.

   For the control, dilute the cells 1:10. Plate 100µl (0.001ng) on LB/ampicillin plates. If using BL21(DE3)pLysS Competent Cells, do not dilute; spread 100µl of these cells directly onto agar plates containing antibiotics.



Calculation of Transformation Efficiency (Colony Forming Units [cfu])

Transformation efficiency is defined as the number of colony forming units (cfu) produced by 1µg of Competent Cells Control DNA (supercoiled plasmid DNA) and is measured by performing a control transformation reaction using a known quantity of DNA, typically 0.1ng, then calculating the number of cfu formed per microgram DNA.

Equation for Transformation Efficiency (cfu/µg)

$$\frac{\text{cfu on control plate}}{\text{ng of Competent Cells Control DNA plated}} \times \frac{1 \times 10^3 \text{ng}}{\mu\text{g}}$$

See additional protocol information in Technical Bulletin #TB095, available online at:
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E. coli Competent Cells: Single-Use Protocol

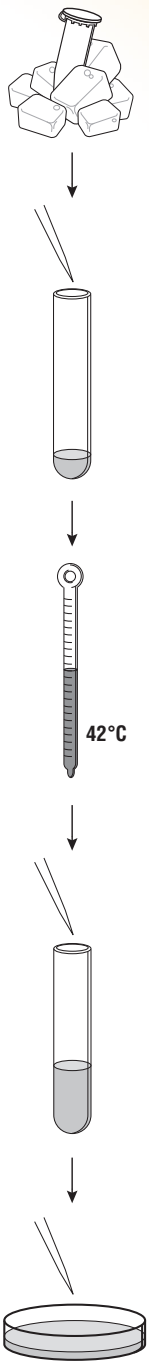
INSTRUCTIONS FOR USE OF PRODUCTS L1195, L2005, L2015 AND L1221.

Quick PROTOCOL

Standard Transformation Protocol for Single-Use Cells

1. Thaw frozen Competent Cells on ice until just thawed.
2. Add 1–50ng of DNA **or** 10pg of Competent Cells Control DNA (supplied as 0.1ng/µl) per 50µl tube of Competent Cells. Quickly flick the tube several times. (Use the Competent Cells Control DNA to determine transformation efficiency.)
3. Immediately return the tubes to ice for 30 minutes.
4. Heat-shock the cells for 15–20 seconds in a water bath at exactly 42°C. **Do not shake.**
5. Immediately place the tubes on ice for 2 minutes.
6. Add 450µl of room temperature SOC medium to each transformation reaction. Incubate for 60 minutes at 37°C with shaking.
7. For each transformation reaction, dilute the cells 1:10 and 1:100. Plate 100µl of the undiluted, 1:10 and 1:100 dilutions onto agar plates containing antibiotics. Incubate the plates at 37°C for 12–14 hours or overnight.

   For the control, dilute the cells 1:4. Plate 100µl (0.001ng) on LB/ampicillin plates. If using BL21(DE3)pLysS Competent Cells, do not dilute; spread 100µl of these cells directly onto agar plates containing antibiotics.



Calculation of Transformation Efficiency (Colony Forming Units [cfu])

Transformation efficiency is defined as the number of colony forming units (cfu) produced by 1µg of Competent Cells Control DNA (supercoiled plasmid DNA) and is measured by performing a control transformation reaction using a known quantity of DNA, typically 0.1ng, then calculating the number of cfu formed per microgram DNA.

Equation for Transformation Efficiency (cfu/µg)

$$\frac{\text{cfu on control plate}}{\text{ng of Competent Cells Control DNA plated}} \times \frac{1 \times 10^3 \text{ng}}{\text{µg}}$$

See additional protocol information in Technical Bulletin #TB095, available online at:
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Printed in USA. Revised 1/13.
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