One Shot™ INVαF´ Chemically Competent *E. coli*

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Contents and storage

Contents	Amount	Storage
One Shot™ INVαF´ Chemically Competent *E. coli*	21 × 50 µL	–80°C
pUC19 vector, supercoiled (10 pg/ µL)	50 µL (in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8)	
SOC medium	6 mL	Room temperature

Genotype

F' *end*A1 *rec*A1 *hsd*R17(r_k^-, m_k^+) *sup*E44 *thi*-1 *gyr*A96 *rel*A1 Φ80*lac*ZΔM15 Δ*lac*(ZYA-argF)U169 λ^-

Materials required but not provided

- 42°C water bath
- Tube rack with space for all transformation reaction tubes (for simultaneous incubation in 42°C water bath)
- 37°C incubator
- 10-cm diameter LB agar plates with appropriate antibiotic
- Ice bucket with ice

Guidelines for transformation

- Be extremely gentle when working with competent cells. Competent cells are highly sensitive to changes in temperature or mechanical lysis caused by pipetting. Start transformation immediately after thawing the cells on ice. Swirl or tap the tube gently to mix reagents; do not mix by pipetting up and down.
- One Shot™ INVαF´ competent cells do not require IPTG to induce expression from the *lac* promoter.
- Perform a transformation control to test the efficiency of the competent cells in the kit. See "Calculate transformation efficiency" for details.

Prepare material for transformation

- Equilibrate a water bath to 42°C.
- Thaw SOC medium, and keep at room temperature.

  Store at room temperature after initial thawing.
- Incubate an appropriate number of 10-cm diameter LB agar plates with antibiotic at 37°C to remove excess moisture (use one plate for each transformation).

  Note: If blue/white screening is required for identification of transformants, spread 40 µL of 40 mg/mL X-Gal in dimethylformamide onto the selective plates. Allow the X-Gal to diffuse into the agar for approximately 1 hour.

Prepare cells

1. Thaw one vial of One Shot™ competent cells on ice for each transformation reaction. **Use the cells immediately**, do not leave them on ice for an extended period of time.
2. Centrifuge the vial(s) containing the ligation reaction(s) briefly and place on ice.
3. Pipet DNA directly into the tube of competent cells.
   - Add 1–5 µL for each ligation mixture. The remaining ligation mixture(s) can be stored at –20°C.
   - Add 1 µL for the pUC19 transformation control.
4. Mix the cells by tapping gently. **Do not** mix by pipetting up and down.
5. Incubate the cells on ice for 30 minutes.

Transform cells

1. Incubate the vial(s) of cells in a 42°C water bath for exactly 30 seconds. Do not mix or shake.
2. Quickly transfer the vial(s) from the 42°C bath and place them on ice.
3. Immediately add 250 µL of SOC medium to each vial of cells.

   SOC is a rich medium; good sterile technique must be practiced to avoid contamination.
4. Place the vial(s) in a microcentrifuge rack on its side in a gyratory shaker-incubator. Secure the rack and tubes with tape to avoid loss of the vial(s).
5. Incubate the vial(s) at 37°C with shaking at 225 rpm for 1 hour.
6. Plate 50–100 µL of cells from each transformation reaction on pre-warmed selective plates, then invert the plates and incubate overnight at 37°C.

   Note: For the pUC19 transformation control, plate 50 µL of cells on a pre-warmed selective plate.
7. Select colonies for further analysis by plasmid isolation, PCR, or sequencing.

Calculate transformation efficiency

Use the following formula to calculate transformation efficiency.

$$\frac{\text{\# of colonies}}{\text{10 pg transformed}} \times \frac{10^6\ \text{pg}}{\mu\text{g}} \times \frac{\text{300 µL transformed cells}}{\text{x µL plated}} = \frac{\text{\# transformants}}{\mu\text{g plasmid DNA}}$$

Limited product warranty

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