Custom Chemically Competent Cells

IMPORTANT!
-80°C Storage Required
Immediately Upon Receipt

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Contents

Components & Storage Conditions

The cells are shipped on dry ice in one container, along with Recovery Medium and supercoiled control pUC19 DNA at 10 pg/µl.

Component	Storage
Custom Competent Cells*	-80°C
Recovery Medium**	-80°C to 20°C
Supercoiled pUC19 DNA (10pg/µl)	-80°C to 20°C

***Competent Cells require immediate storage at –80° C**.
**Additional Recovery Medium is available for purchase (cat. no. 80026-1 (8 x 12ml))

Preparation for Transformation

Quality testing of chemically competent cells is performed using 40 µl of competent cells per transformation in a 17 mm x 100 mm culture tube. Optimal settings for heat shock are listed in the table below.

Optimal Setting		Alternate Settings (~ 20-50% lower efficiencies)
17 mm x 100 mm 42°C for 45 seconds		1.5ml microcentrifuge tube 42°C for 30 seconds 42°C for 60 seconds

Transformation control reactions are performed with 1 µl (10 pg) of supercoiled pUC19 DNA.
Transformation is performed by heat shock at 42 °C, followed by incubation on ice.
To ensure successful transformation results, the following precautions must be taken:

- Ligation reactions performed with Lucigen's CloneDirect™ Ligation Buffer (included with Lucigen's Cloning or Ligation Kits) must be heat killed at 70ºC for 15 minutes before transformation. The ligation reaction can be used immediately after heat inactivation, without purification of the ligation products.
- Culture tubes must be thoroughly pre-chilled on ice before use.
- The cells must be completely thawed **on ice** before use.
- For highest transformation efficiency, use the provided Recovery Medium to resuspend the cells after electroporation. Use of TB, SOC, or other media will result in lower transformation efficiencies.

Transformation Protocol

1. Chill sterile culture tubes on ice (17 mm x 100 mm tubes, one tube for each transformation reaction).

2. Remove cells from the -86°C freezer and thaw completely on wet ice (10-20 minutes).
3. Add 40 µl of cells to the chilled culture tube.

4. Add 1 µl of ligation reaction or DNA sample to the 40 µl of cells on ice. (Failure to purify or heat-inactivate the ligation reaction may prevent transformation.) Stir briefly with pipet tip; **do not** pipet up and down to mix, which can introduce air bubbles and warm the cells.

5. Incubate on ice for 30 minutes.

6. Heat shock cells by placing them in a 42 ^{o}C water bath for 45 seconds.

7. Return the cells to ice for 2 minutes.

8. Add 960 µl of room temperature Recovery Medium to the cells in the culture tube.

9. Place the tubes in a shaking incubator at 250 rpm for 1 hour at 37 ^{o}C.

10. Plate up to 100 µl of transformed cells on YT agar plates containing the appropriate antibiotic. Note: the quality of LB plates varies widely. Transformants plated on LB may grow slowly.

11. Incubate the plates overnight at 37°C.

12. Transformed clones can be further grown in TB or any other rich culture medium.