Lucigen®
Simplifying Genomics

Endura™ Chemically Competent and Electrocompetent Cells

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Technical Support

Lucigen is dedicated to the success and satisfaction of our customers. Our products are tested to assure they perform as specified when used according to our recommendations. It is imperative that the reagents supplied by the user, especially the DNA targets to be cloned, are of the highest quality. Please follow the manual carefully and contact our technical service representatives if additional information is necessary. We encourage you to contact us with your comments regarding the performance of our products in your applications. Thank you.

Lucigen Technical Support

Email: techsupport@lucigen.com
Phone: (888) 575-9695

Product Guarantee: Lucigen guarantees that this product will perform as specified for one year from the date of shipment. Please avoid using reagents for greater than one year from receipt.

Components & Storage Conditions

Lucigen's Endura Competent Cells are packed in Lucigen's DUO format for your convenience:

Electrocompetent cells:
DUO: 50 µL per vial, enough for two 25-µL reactions
Chemically competent cells:
DUO: 80 µL per vial, enough for two 40-µL reactions
SOLO: 40 µL per vial, enough for one reaction

The cells are shipped on dry ice in one container, along with Recovery Medium and supercoiled control pUC19 DNA at 10 pg/µl. Please refer to Table 1 for a compete listing of Endura Electrocompetent Cells, efficiencies, and catalog numbers.

Competent cells require storage at –80 ºC.

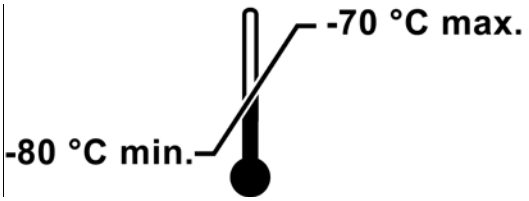

Table 1: **Endura Competent Cells available from Lucigen**

STRAIN	Format	Efficiency (cfu/µg pUC19)	Transformations		Catalog #	Storage
Endura Chemically Competent Cells (Blue cap)	SOLO	> 1 x 10^7	12 24	(12 x 40 µL) (24 x 40 µL)	60241-1 60241-2	**-80 ºC**
	DUO	> 1 x 10^7	4 12 24	(2 x 80 µL) (6 x 80 µL) (12 x 80 µL)	60240-0 60240-1 60240-2	**-80 ºC**
Endura Electrocompetent Cells (Yellow cap)	DUO	> 1 x 10^{10}	4 12 24	(2 x 50 µL) (6 x 50 µL) (12 x 50 µL)	60242-0 60242-1 60242-2	**-80 ºC**
*Recovery Medium	-		4 12 24 	(4 x 1 mL) (1 x 12 mL) (2 x 12 mL) (8 x 12 mL)	---- ---- ---- 80026-1	**-80 to +20 ºC**
**Supercoiled pUC19 DNA (10 pg/µL)	-			(1 x 20 µL)	----	**-80 to -20 ºC**

* **Transformation of Endura Electrocompetent Cells with GeCKO lentiviral libraries may require extra Recovery Medium.** The Zhang lab protocol (available from Addgene) recommends ~2 mL of Recovery Medium per transformation. Lucigen Endura kits contain 1 mL of Recovery Medium per transformation.

**Provided as a positive control for transformation. pUC19 contains ampicillin resistance.

Endura Competent Cells

Lucigen's Endura Competent Cells are noted for their ability to clone and maintain plasmid DNA sequences that are otherwise unstable, such as those with inverted repeats or sequences that are otherwise prone to undesired recombination events.

Such unstable inserts are commonly found in retroviral DNA, and can prove exceptionally difficult to isolate and propagate in traditional cloning strains. Lucigen's Endura Competent Cells are available in high efficiency chemically competent or electrocompetent versions to make difficult cloning work more reliable and efficient.

Transformation Efficiency:
Chemically Competent Cells: $\geq$ 1 x 10^7 cfu/µg
Electrocompetent Cells: $\geq$ 1 x 10^{10} cfu/µg

Genotype

recA13 supE44 ara-14 galK2 lacY1 proA2 rpsL20 (Str^R) xyl-5 λ$^-$ leu mtl-1 F$^-$ mcrB mrr hsdS20(r_B^-, m_B^-)

Usage Guidelines: Electrocompetent Cells

Note: Transformation of Endura Electrocompetent Cells with GeCKO lentiviral libraries may require extra Recovery Medium. The Zhang lab protocol (available from Addgene) recommends ~2 mL of Recovery Medium per transformation. Lucigen Endura kits contain 1 mL of Recovery Medium per transformation. See Table 1 for ordering information.

Preparation for Transformation

Transformation is carried out in a 0.1 cm gap cuvette using 25 µL of Endura Electrocompetent Cells. Optimal settings for electroporation are listed in the table below. Typical time constants are 3.5 to 4.5 msec.

Optimal Setting		Alternate Settings (~ 20-50% lower efficiencies)
1.0 mm cuvette 10 µF 600 Ohms 1800 Volts		1.0 mm cuvette 25 µF 200 Ohms 1400 – 1600 Volts

Suggested Electroporation Systems:
Bio-Rad Micro Pulser #165-2100; Bio-Rad E. coli Pulser #165-2102; Bio-Rad Gene Pulser II #165-2105; BTX ECM630 Electroporation System; Eppendorf Electroporator 2510. Optional transformation control reactions include electroporation with 1 µL (10 pg) of supercoiled pUC19 DNA.

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 70 ºC for 15 minutes before transformation. The ligation reaction can be used directly after heat inactivation, without purification of the ligation products.
- DNA samples in other buffers must be purified and dissolved in water or a buffer with low ionic strength, such as TE. The presence of salt in the electroporation sample leads to arcing at high voltage, resulting in loss of the cells and DNA.
- Microcentrifuge tubes and electroporation cuvettes must be thoroughly pre-chilled on ice before use. Successful results can be obtained with cuvettes from many sources including BTX (Model 610), Eppendorf (Cat. #940001005), and BioRad (Cat. #165-2089).
- 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 SOC or other media will result in lower efficiencies.
- Prepare nutrient agar plus appropriate antibiotic. Low salt media, such as LB Lennox, is recommended. High salt media such as LB Miller may result in variations in colony size.

Transformation Protocol

1. Have Recovery Medium and 17 mm x 100 mm sterile culture tubes readily available at room temperature (one tube for each transformation reaction). Transformation efficiency may decrease with the use of SOC or other media.
2. Place electroporation cuvettes (0.1 cm gap) and microcentrifuge tubes on ice (one cuvette and one microfuge tube for each transformation reaction).
3. Remove cells from the -80 °C freezer and place on wet ice until they thaw **completely** (10-20 minutes).
4. When cells are thawed, mix them by tapping gently. Aliquot 25 µL of cells to the chilled microcentrifuge tubes on ice. (Omit this step if using *E. cloni* SOLOs, which contain 25 µL of cells per tube).
5. If using ligation buffer from any Lucigen Cloning Kit, add 1 µL of the heat-denatured ligation reaction to the 25 µL of cells on ice. Failure to heat-inactivate the ligation reaction will prevent transformation. Stir briefly with pipet tip; **do not** pipet up and down to mix, which can introduce air bubbles and warm the cells. Use of more than 2 µL of ligation mix may cause electrical arcing during electroporation.

   For ligation reactions using other commercial kits, please refer to the manufacturer's instructions.
6. Carefully pipet 25 µL of the cell/DNA mixture into a chilled electroporation cuvette without introducing bubbles. Quickly flick the cuvette downward with your wrist to deposit the cells across the bottom of the well. Electroporate according to the conditions recommended above.
7. Within 10 seconds of the pulse, add 975 µL of Recovery Medium to the cuvette and pipet up and down three times to resuspend the cells. Transfer the cells and Recovery Medium to a culture tube.
8. Place the tube in a shaking incubator at 250 rpm for 1 hour at 37 °C.
9. Spread up to 100 µL of transformed cells on nutrient agar plates containing the appropriate antibiotic.
10. Incubate the plates overnight at 37 °C.
11. Transformed clones can be further grown in any rich culture medium.

Usage Guidelines: Chemically Competent Cells

Preparation for Transformation

To ensure successful transformation results, the following precautions must be taken:

- For best results, Lucigen CloneSmart® ligation reactions must be heat killed at 70 ºC for 15 minutes before transformation. Alternately, the reactions may be purified, if desired. For other ligation reactions, follow the manufacturer's recommendations.
- Prepare nutrient agar plus antibiotic for selection. Low salt media, such as LB Lennox, is recommended. High salt media such as LB Miller may result in variations in colony size.
- All microcentrifuge 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 transformation. Use of SOC or other media will result in lower efficiencies.

Transformation Protocol

1. Prepare nutrient agar (e.g., LB) plates with antibiotic for selection.
2. Chill sterile culture tubes on ice (17 mm x 100 mm tubes, one tube for each transformation reaction).
3. Remove cells from the -80 °C freezer and thaw completely on wet ice (10-20 minutes).
4. Add 40 µL of cells to the chilled culture tube.
5. Add 1-4 µl of heat-inactivated ligation reaction or DNA sample to the 40 µL of cells on ice. (Failure to heat-inactivate—70 °C for 15 minutes—or otherwise purify, 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.
6. Incubate on ice for 30 minutes.
7. Heat shock cells by placing them in a 42 °C water bath for 45 seconds.
8. Return the cells to ice for 2 minutes.
9. Add 960 µL of room temperature Recovery Medium to the cells in the culture tube. When using these cells with a cloning kit, follow the Recovery Medium volume given in that kit manual.
10. Place the tubes in a shaking incubator at 250 rpm for 1 hour at 37 °C.
11. Plate up to 100 µL of transformed cells on nutrient agar plates containing the appropriate antibiotic. Note: the quality of LB plates varies widely. Transformants plated on LB may grow slowly.
12. Incubate the plates overnight at 37 °C.
13. Transformed clones can be further grown in any rich culture medium (e.g., LB, or TB).

Media Recipes

LB Lennox Agar Plates

Per liter:
- 10 g tryptone
- 5 g yeast extract
- 5 g NaCl
- 15 g agar

Medium for Growth of Transformants
LB Miller

Per liter:
- 10 g tryptone
- 5 g yeast extract
- 10 g NaCl

Add all components to deionized water. Adjust pH to 7.0 with NaOH. Autoclave and cool to 55 °C.

TB

Per liter:
- 11.8 g tryptone
- 23.6 g yeast extract
- 9.4 g dipotassium hydrogen phosphate (anhydrous)
- 2.2 g potassium dihydrogen phosphate (anhydrous)
- 0.4% glycerol

Add all components to deionized water. Autoclave and cool to 55 °C.

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