Lucigen®
Simplifying Genomics

# *E. cloni*® 5-alpha Chemically Competent Cells

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE


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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 are of the highest quality. Please follow the instructions 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: techserv@lucigen.com

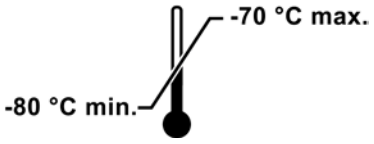
Phone: (888) 575-9695

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 *E. cloni* 5-alpha Chemically Competent Cells yield ≥1 x $10^8$ cfu/μg pUC19 DNA. The cells are shipped on dry ice in one container, with supercoiled control pUC19 DNA at 10 pg/ μL,and Recovery Medium. *E. cloni* 5-alpha Chemically Competent Cells are available in 100- μL aliquots (DUOs), sufficient for two transformations per tube.

**All competent cells require storage at -80 °C**



### *E. cloni* 5-alpha Chemically Competent Cells:

| STRAIN | Efficiency (cfu/μg pUC19) | Transformations | Catalog # | Storage |
|---|---|---|---|---|
| *E. cloni* 5-alpha Chemically Competent DUOs (Green cap) | ≥ 1 x $10^8$ | 12 (6 x 100 μL)<br>24 (12 X 100 μL) | 60602-1<br>60602-2 | **-80 °C** |
| Recovery Medium | | 12 (1 x 12 mL)<br>24 (2 x 12 mL)<br>96 (8 x 12 mL) | ----<br>----<br>80026-1 | **-20 to -80 °C** |
| Supercoiled pUC19 DNA (10 pg/ μL) | | (1 x 20 μL) | ---- | **-20 to -80 °C** |

## Description & Uses

*E. cloni* 5-alpha Chemically Competent Cells are versatile and useful in a wide variety of applications including routine cloning, subcloning, and plasmid isolation with or without blue/white screening. They can directly replace commonly used cloning strains like DH5α. They give high yield and high quality plasmid DNA due to the *end*A1 and *rec*A1 mutations. They contain the wildtype *mcr* and *mrr* alleles, so they are NOT recommended for direct cloning of methylated genomic DNA; instead, Lucigen's *E. cloni* 10G Competent Cells should be used in these cases.

### *E. cloni* 5-alpha Genotype:

*fhuA2*Δ*(argF-lacZ)U169 phoA glnV44* Φ*80* Δ*(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*

### Transformation Control

As a control for transformation, *E. cloni* 5-alpha Chemically Competent Cells are provided with supercoiled pUC19 DNA at a concentration of 10 pg/ μL –use 1 μL for transformation. Plate pUC19 transformants on plates containing ampicillin or carbenicillin.

## Preparation for Transformation

*E. cloni* 5-alpha Chemically Competent Cells are provided in aliquots of 100 μL (two transformations). Use 50 μL per transformation. 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:**

- 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.
- 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.

## Transformation Protocol

1. Prepare nutrient agar plates with appropriate antibiotic.
2. Chill sterile culture tubes on ice (17 mm x 100 mm tubes, one tube for each transformation reaction).
3. Remove *E. cloni* 5-alpha cells from the -80 °C freezer and thaw completely on wet ice (10-20 minutes).
4. Add 50 µL of the cells to the chilled culture tube.
5. Add 1-4 µL of ligation reaction or DNA sample to the 50 µL of cells on ice. (Failure to purify or heat-inactivate, 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 30 seconds.
8. Return the cells to ice for 2 minutes.
9. Add 950 µL of room temperature Recovery Medium to the cells in the culture tube. When using these cells with a Lucigen 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.
12. Incubate the plates overnight at 37 °C.
13. Transformed clones can be further grown in any rich culture medium.

### 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.