

Please note: The concentration of the included pUC19 has been changed from 1ng/μl to 10pg/μl. Do not dilute the plasmid before performing the transformation positive control. Please contact Lucigen if you have any questions.

***E. cloni*[®] 5-alpha Chemically Competent Cells**

**IMPORTANT!
-80°C Storage Required
Immediately Upon Receipt**

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E. cloni[®] 5-alpha Chemically Competent Cells

Contents

| | |
|---|---|
| Components & Storage Conditions..... | 3 |
| <i>E. cloni</i> 5-alpha Chemically Competent Cells..... | 3 |
| Preparation for Transformation | 4 |
| Transformation Protocol..... | 4 |
| Related Products..... | 5 |

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E. cloni[®] 5-alpha Chemically Competent Cells

Components & Storage Conditions

Lucigen's *E. cloni* 5-alpha Chemically Competent Cells yield $\geq 1 \times 10^8$ cfu/ μ g pUC19 DNA. The cells are shipped on dry ice in one container, with supercoiled control pUC19 DNA at 10 pg/ μ l, and Recovery Medium. *E. cloni* 5-alpha Chemically Competent Cells are available in 100- μ l aliquots (DUOs), sufficient for two transformations per tube.

E. cloni 5-alpha Chemically Competent Cells:

Store at -80°C

| STRAIN | Efficiency (cfu/ μ g pUC19) | Transformations | Catalog # | Storage |
|---|---------------------------------|---|-------------------------|------------------------------|
| <i>E. cloni</i> 5-alpha Chemically Competent DUOs | $\geq 1 \times 10^8$ | 12 (6 x 100 μ l) 24 (12 X 100 μ l) | 60602-1 60602-2 | -80°C |
| Recovery Medium | | 12 (1 x 12 mls) 24 (2 x 12 mls) 96 (8 x 12 mls) | ---- ---- 80026-1 | -20 to -80°C |
| Supercoiled pUC19 DNA (10 pg/ μ l) | | (1 x 20 μ l) | ---- | -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 DH5 α . They give high yield and high quality plasmid DNA due to the *endA1* and *recA1* 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/ μ l—use 1 μ l for transformation. Plate pUC19 transformants on plates containing ampicillin or carbenicillin.

E. cloni[®] 5-alpha Chemically Competent Cells

Preparation for Transformation

E. cloni 5-alpha Chemically Competent Cells are provided in aliquots of 100 µl (two transformations). Use 50 µl per transformation. Refreeze any unused cells at -80 °C. 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°C 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.

Related Lucigen Products

- CloneSmart[®] Blunt Cloning Kits
- DNATerminator[®] End Repair Kits
- PCRTerminator[®] End Repair Kits
- UltraClone[™] DNA Ligation & Transformation Kits
- CloneDirect[™] Rapid Ligation Kits
- ClonePlex[®] Library Construction Kits
- pEZSeq[™] Blunt Cloning Kits
- cSMART[™] cDNA Cloning Kits
- *E. coli*[®] Electrocompetent Cells
- OverExpress[™] Competent Cells