

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*[®] 96-well 10G**

Chemically Competent Cells

IMPORTANT!
-80°C Storage Required
Immediately Upon Receipt

Lucigen[®] Corporation
Advanced Products for Molecular Biology

2120 W. Greenview Drive
Middleton, WI 53562 USA
Toll Free: (888) 575-9695
Phone: (608) 831-9011
FAX: (608) 831-9012
E-mail: lucigen@lucigen.com
Internet: www.lucigen.com

E. cloni[®] 96-well 10G Chemically Competent Cells

Contents

Components & Storage Conditions.....	3
<i>E. cloni</i> 10G Chemically Competent Cells	3
Preparation for Transformation	4
Transformation Protocol.....	4
Related Products.....	5

Notice of Limited Label License, Copyright, Patents, Warranties, Disclaimers and Trademarks

Copyright© 2001-2010 by Lucigen Corp. All rights reserved. Lucigen, BigEasy, ClonePlex, CloneSmart, CopyRight, DNATerminator, *E. cloni*, EconoTaq, ExCyto, Lucigen, NanoClone, PCR Terminator, pJAZZ, pSMART, pTrueBlue, and PyroPhage are registered trademarks of Lucigen Corporation. bSMART, Blue/White cloning without the blues, ChimeraFree, CloneDirect, cSMART, eLucidations, GapFree, PCR SMART, pEZ, pEZSeq, pGC, pLEXX-AK, pRANGER, Replicator, Softag, The Cloning Company, The Molecular Cloning Company, Transformance, TSA, and UltraClone are trademarks of Lucigen Corporation. Hydroshear and GeneMachines are trademarks of Genomic Solutions, Inc. DH10B is a trademark of Invitrogen Corp.

Lucigen's products are sold for research use only and are not to be used in humans or for medical diagnostics. Lucigen's liability with respect to any of its products is limited to the replacement of the product. No other warranties of any kind, expressed or implied, including without limitation, any implied fitness for any particular use, are provided by Lucigen. Lucigen is not liable for any direct, indirect, incidental or consequential damages arising out of or in connection with the use or inability to use any of its products.

If the purchaser is not willing to accept these use limitations, Lucigen Corporation is willing to accept return of the product for a full refund. For information on obtaining a license, contact Lucigen Corporation, 2120 W. Greenview Dr., Middleton, WI 53562. Email: Lucigen@lucigen.com. Phone: 608-831-9011. Fax 608-831-9012.

E. cloni[®] 96-well 10G Chemically Competent Cells

Components & Storage Conditions

Lucigen's *E. cloni* 10G Chemically Competent Cells yield $\geq 1 \times 10^8$ cfu/ μ g pUC19. The cells are shipped on dry ice in one container, along with supercoiled control pUC19 DNA at 10 pg/ μ l, and Recovery Medium. Please refer to the table below for catalog numbers.

E. cloni[®] 96-well 10G Chemically Competent Cells: Store at **-80° C**

STRAIN	Catalog Number	Number of Plates	Transformations	Storage
<i>E. cloni</i> 96-well 10G Chemically Competent Cells	60096-1	1	96 (96 x 20 μ l)	-80°C
	60096-4	4	384 (96 x 20 μ l)	-80°C

Additional Included Components:

Component	With 1 Plate	With 4 Plates	Storage
Recovery Medium	1 (1 x 12 ml)	4 (4 x 12 ml)	-20 to -80°C
pUC19 DNA (10 pg / μ l)	1 (1 x 20 μ l)	1 (1 x 20 μ l)	-20 to -80°C
Plate Cover	1 Cover	4 Covers	Ambient
Strip Caps	1 Bag (12 strips)	4 Bags (4 x 12 strips)	Ambient

E. cloni 10G Chemically Competent Cells

E. cloni 10G Chemically Competent Cells are a strain of *E. coli* that have been optimized for high efficiency transformation by heat shock, producing $\geq 1 \times 10^8$ cfu/ μ g supercoiled pUC19 DNA. These cells are ideal for cloning and propagation of plasmid, cosmid, or fosmid clones. They can directly replace commonly used cloning strains like DH10B.

The *E. cloni* 10G strain contains inactive *mcr* and *mrr* alleles, allowing methylated genomic DNA isolated directly from mammalian or plant cells to be cloned without deletions or rearrangements. The cells give high yield and high quality plasmid DNA due to the *endA1* and *recA1* mutations. They are bacteriophage T1-resistant (*tonA* mutation). The *rpsL* mutation confers resistance to streptomycin.

E. cloni 10G Genotype:

F⁻ *mcrA* Δ (*mrr-hsdRMS-mcrBC*) *endA1 recA1* ϕ 80/*lacZ* Δ M15 Δ *lacX74 araD139* Δ (*ara, leu*)7697 *galU galK rpsL nupG* λ ⁻ *tonA*

Transformation Control

As a control for transformation, *E. cloni* Competent Cells are provided with supercoiled pUC19 DNA at a concentration of 10 pg/ μ l. Use 1 μ l (10 pg) for transformation. Plate pUC19 transformants on plates containing ampicillin or carbenicillin.

E. cloni[®] 96-well 10G Chemically Competent Cells

Preparation for Transformation

E. cloni 96-well Chemically Competent Cells are provided in aliquots of 20 µl per well. Transformation is performed by heat shock at 34°C, followed by incubation on ice. The 96-well plates can be divided into four 24-well segments (3 x 8 wells) by cutting or breaking along the plate perforation. Before dividing the plate, the foil seal should be scored with a razor blade along the perforation to avoid opening unneeded wells. For transformation, the foil seal can be perforated with a pipet tip or removed entirely.

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 (e.g., LB) plates with antibiotic for selection.
- 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 (e.g., LB) plates with antibiotic for selection.
2. Preheat a heat block to 34°C or set a 96-well thermal cycler to hold temperature at 34°C. Alternately, a water bath set a 34° C can be used but efficiency may drop up to ten fold.
3. Remove *E. cloni* cells from the -80°C freezer and thaw completely on wet ice (5-10 minutes). Before adding the DNA sample, verify that the cells have completely thawed by gently tapping the plate.
4. Use one of the following methods to add the DNA sample to the cells. Take care to make sure the DNA is added directly to the cells and not to the side of the wells.
 - a. Using a pipette tip, pierce the foil seal, add 1 µl of the DNA sample to the cells, and stir briefly with the pipet tip. **Do not** pipet up and down to mix, which can introduce air bubbles and warm the cells. Cover the wells with either the provided 8-cap strips or plate lid to protect from contamination.

or

 - b. Peel the foil seal off of the plate, add 1 µl of the DNA sample to each well, and stir briefly with the pipet tip. **Do not** pipet up and down to mix, which can introduce air bubbles and warm the cells. Cover the wells with either the provided 8-cap strips or plate lid to protect from contamination.
5. Incubate on ice for 5 minutes.
6. Heat shock cells by placing them at 34°C in a heat block or thermal cycler for 30 seconds.
7. Return the cells to ice for 2 minutes.
8. Add 80 µl of room temperature Recovery Medium to each well of cells.
9. Place the plate in an incubator for 1 hour at 37°C. Shaking is not necessary for cell growth.
10. Plate up to 50 µl of transformed cells on nutrient agar plates containing the appropriate antibiotic.
11. Incubate the plates overnight at 37°C.
12. 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. cloni*[®] Electrocompetent Cells
- OverExpress[™] Competent Cells