LGC

Manual

E. cloni 96-well Chemically Competent Cells

For Research Use Only. Not for use in diagnostic procedures.

IMPORTANT
-80 ºC storage required
immediately upon receipt

BIOSEARCH™
TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

Manual

E. cloni 96-well Chemically Competent Cells

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Technical support

LGC, Biosearch Technologies™ 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.

Technical Support
Email: techsupport@lgcgroup.com

Product guarantee: Biosearch Technologies 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.

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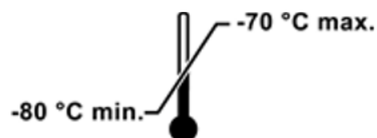
E. cloni 96-well Chemically Competent Cells

Components and storage conditions

Biosearch Technologies' E. cloni 10G Chemically Competent Cells yield > 1 x 10^8 cfu/µg pUC19. The cells are shipped on dry ice in one container, along with supercoiled control pUC19 DNA at 10 pg/µL, and Recovery Medium. Please refer to the table below for catalog numbers.



E. cloni 96-well 10G Chemically Competent Cells:

Strain	Catalog number	Number of plates	Transformations	Storage
E. cloni 96-well 10G Chemically Competent Cells	60096-1	1	96 (96 x 20 µL)	-80 °C
	60096-4	4	384 (96 x 20 µL)	-80 °C

Additional Included Components:

Component	With 1 plate	With 4 plates	Storage
Recovery medium	1 x 12 mL	4 x 12 mL	-20 to -80 °C
pUC19 DNA (10 pg/µL)	20 µL	20 µL	-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 optimised for high efficiency transformation by heat shock, producing > 1 × 10^8 cfu/µg 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 *end*A1 and *rec*A1 mutations. They are bacteriophage T1-resistant (*ton*A mutation). The *rps*L mutation confers resistance to streptomycin.

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E. cloni 96-well Chemically Competent Cells

E. cloni 10G Genotype:
F¯ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) *end*A1 *rec*A1 ϕ80d*lac*ZΔM15 ΔlacX74 araD139 Δ(*ara,leu*)7697 *gal*U *gal*K *rps*L (Str^R) *nup*G λ- *ton*A

Cell line	Supports cloning methylated DNA	Contains F-plasmid	Suitable for blue/white screening	IPTG required for blue/white screening
E. cloni 10G	Yes	No	Yes	No

Transformation control
As a control for transformation, E. cloni 96-well Chemically Competent Cells are provided with supercoiled pUC19 DNA at a concentration of 10 pg/µL. Use 1 µL (10 pg) for transformation. Plate pUC19 transformants on plates containing ampicillin or carbenicillin.

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 × 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:

- If you are using a commercially available ligation kit to assemble your ligation reactions (such as the Biosearch FastLink(TM) DNA Ligation Kit), follow the manufacturer's recommendations for heat-killing or purifying the reactions before transformation.
- Prepare nutrient agar plus antibiotic for selection. Use LB-Lennox agar to achieve optimal cell growth.
- 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 ºC or set a 96-well thermal cycler to hold temperature at 34 °C. Alternately, a water bath set a 34 ºC 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.

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 deionised 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 deionised water. Autoclave and cool to 55 °C.

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