CJ236 Electrocompetent Cells

Cat#: ECC1311-01 | Format: 20 x 50 µl | Storage: -80°C

Description

CJ236 Electrocompetent Cells are an E. coli host strain prepared for electroporation-based transformation. The CJ236 strain is deficient in dUTPase (dut^-) and uracil DNA glycosylase (ung^-), which causes newly synthesized DNA to incorporate deoxyuracil (dU) in place of thymine (T). This uracil-substituted single-stranded DNA (ssDNA) serves as a template for high-efficiency Kunkel-method site-directed mutagenesis.

CJ236 harbors an F-factor with a chloramphenicol resistance marker (CamR), enabling phage infection for M13 ssDNA production. The strain was derived from BW313 by introducing pCJ105, an F' CmR construct. CJ236 electrocompetent cells are suitable for preparing uracil-containing M13 and phagemid templates for the Kunkel mutagenesis workflow.

Electrocompetent cells are prepared to take up foreign DNA upon exposure to a brief high-voltage electrical pulse (electroporation). The electric field transiently permeabilizes the cell membrane, allowing plasmid DNA or other constructs to enter the cell. Compared to chemically competent cells, electrocompetent cells typically yield higher transformation efficiencies, making them suitable for demanding applications such as library construction and low-copy plasmid transformation.

Genotype

FΔ(HindIII)::cat (Tra^+ Pil^+ CamR) / ung-1 relA1 dut-1 thi-1 spoT1 mcrA

Specifications

Catalog Number	Size	Transformation Efficiency	Storage
ECC1311-01	20 x 50 µl	≥1 x 10^{10} cfu/µg pUC19	-80°C

General Handling

Electrocompetent cells are highly sensitive to temperature changes and mechanical disruption. Follow these precautions to preserve transformation efficiency:

- Keep cells on ice at all times. Never allow them to warm above 0°C before electroporation.
- Thaw cells slowly on ice (approximately 10 minutes). Do not thaw at room temperature.
- Add DNA immediately after thawing. Mix by gently tapping or flicking the tube. Do not pipet or vortex.
- Use only DNA dissolved in water or TE buffer. Salt in the DNA solution will cause arcing during electroporation.
- Chill the electroporation cuvette on ice before use.
- Work quickly between electroporation and adding recovery medium to maximize viability.

Materials Required but Not Supplied

- Electroporator (e.g., Bio-Rad Gene Pulser or equivalent)
- 1 mm gap electroporation cuvettes (pre-chilled on ice)
- 37°C shaking incubator
- 37°C water bath or heat block
- SOC medium

- LB agar plates (10 cm diameter) with appropriate selective antibiotic
- Microcentrifuge tubes (pre-chilled on ice)
- Ice bucket with ice

Before Starting

- Set the electroporator to 1.7–2.5 kV (optimize for strain), 200 Ω, and 25 µF.
- Pre-warm SOC medium to 37°C.
- Pre-warm LB agar plates with appropriate antibiotic at 37°C.
- Place microcentrifuge tubes and 1 mm electroporation cuvettes on ice to chill.
- For a transformation efficiency control, dilute pUC19 to 10 pg/µl in deionized water.

Electroporation Protocol

1. Thaw one tube of electrocompetent cells on ice for approximately 10 minutes.
2. Flick the tube gently to mix. Transfer 25 µl of cells to a pre-chilled microcentrifuge tube.
3. Add 1 µl of DNA solution (10 pg/µl in deionized water) to the cells. Mix by gently flicking. Do not pipet or vortex.
4. Transfer the cell-DNA mixture to a pre-chilled 1 mm electroporation cuvette. Tap the cuvette on the bench twice to settle the liquid. Wipe the exterior dry.
5. Place the cuvette in the electroporation chamber. Apply a single pulse (do not hold the button). A time constant of 4–5 ms indicates a successful pulse.
6. Immediately add 975 µl of pre-warmed SOC medium to the cuvette. Mix by pipetting up and down once. Transfer to a 15 ml tube.
7. Incubate at 37°C with rotation or shaking for 1 hour.
8. For efficiency controls using 10 pg pUC19, prepare two dilutions and plate 100 µl each: • 1,000-fold dilution: 10 µl cells + 990 µl SOC • 100-fold dilution: 100 µl cells + 900 µl SOC
9. Plate onto LB agar with appropriate antibiotic. Incubate overnight at 37°C.

Storage and Stability

Store at -80°C. Do not store at -20°C. Avoid repeated freeze-thaw cycles, as each cycle significantly reduces transformation efficiency. If reduced efficiency is suspected, verify performance using pUC19 control plasmid before use in critical applications.

Notes

- CJ236 is intended for the preparation of uracil-substituted ssDNA templates for Kunkel-method site-directed mutagenesis. It is not recommended as a general-purpose cloning host.
- The F-factor enables phage infection required for M13 ssDNA production. Maintain chloramphenicol selection when propagating the strain to preserve the F-factor.
- Optimal electroporation voltage may vary by instrument. Optimize within the 1.7–2.5 kV range if efficiency is suboptimal.
- Salt contamination in the DNA sample is the most common cause of arcing. Ensure DNA is dissolved in water or low-salt buffer and that the cuvette exterior is dry before pulsing.

References

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- Kunkel TA, Bebenek K, and McClary J. (1991) Methods Enzymol. 204:125–139. Academic Press, San Diego.

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