CJ236 Chemically Competent Cells

Cat#: CC1321-01 | Format: 20 x 50 µl | Storage: -80°C

Description

CJ236 Chemically Competent Cells are an E. coli host strain used to generate uracil-containing single-stranded DNA (ssDNA) for Kunkel-method site-directed mutagenesis. In CJ236, a portion of the thymine (T) residues in newly synthesized DNA is replaced with deoxyuracil (dU) due to deficiency in dUTPase (dut^-) and uracil DNA glycosylase (ung^-). This uracil-substituted ssDNA serves as a template for mutagenesis with high efficiency.

CJ236 harbors an F-factor carrying 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 is suitable for preparing uracil-containing M13 and phagemid templates for use in the Kunkel mutagenesis workflow.

Genotype

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

Specifications

Catalog Number	Size	Transformation Efficiency	Storage
CC1321-01	20 x 50 µl	1 x 10^7 cfu/µg pUC19	-80°C

Materials Required but Not Supplied

- 37°C shaking incubator and non-shaking incubator
- 42°C water bath
- LB agar plates (10 cm diameter) with appropriate selective antibiotic
- SOC medium
- Ice bucket with ice

Before Starting

- Equilibrate the water bath to 42°C.
- Pre-warm SOC medium to 37°C.
- Prepare LB agar plates with the appropriate antibiotic for selection.

Transformation Protocol

1. Thaw one tube of competent cells on ice (approximately 10 minutes).
2. Add 1–2 µl of plasmid DNA directly to the competent cells. Mix gently by tapping or swirling. Do not vortex.
3. Incubate on ice for 20–30 minutes.
4. Heat shock at 42°C in a water bath for exactly 45 seconds.
5. Return the tube to ice immediately and incubate for 2 minutes.
6. Add 250 µl of pre-warmed SOC medium. Incubate at 37°C with shaking for 1 hour.
7. Plate the entire transformation onto an LB agar plate containing the appropriate antibiotic.
8. Incubate the plate at 37°C for 12–15 hours.

Storage and Stability

Store at -80°C. Do not store at -20°C. Repeated freeze-thaw cycles or storage above -80°C will reduce 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 mutagenesis. It is not recommended as a general-purpose cloning host.
- The F-factor in CJ236 enables phage infection required for M13 ssDNA production. Maintain chloramphenicol selection when propagating the strain to preserve the F-factor.
- Cell density upon receipt: 1–2 x 10^9 bacteria/ml.

References

- Joyce CM and Grindley ND. (1984) J. Bacteriol. 158:636–643.
- Raleigh EA, Lech K, and Brent R. (1989) In: Ausubel FM et al. (Eds.), Current Protocols in Molecular Biology. Wiley, New York.
- Kunkel TA, Bebenek K, and McClary J. (1991) Methods Enzymol. 204:125–139. Academic Press, San Diego.
- Kunkel TA et al. (1987) Methods Enzymol. 154:367–382. Academic Press, San Diego.

Biomiga, Inc. | 10637 Roselle Street, Suite C, San Diego, CA 92121 | Tel: (858) 603-3219 | info@biomiga.com | www.biomiga.com