

# *E. coli* K12 CJ236



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E4141S 003140619061

## E4141S

Lot: 0031406 Exp: 6/19 Store at -20°C

**Description:** A suspension of *E. coli* strain CJ236 which has been grown in LB medium and brought to 50% glycerol.

**Genotype:** F $\Delta$ (*HindIII*)::cat (Tra<sup>+</sup> Pil<sup>+</sup> Cam<sup>R</sup>)/ *ung-1 relA1 dut-1 thi-1 spoT1 mcrA*

**Use of Strain CJ236:** CJ236 is *dut<sup>-</sup> ung<sup>-</sup>* and contains an F-factor carrying a selectable marker (chloramphenicol resistance). It was made by taking an isolate of BW313 that had spontaneously become F<sup>-</sup> and introducing pCJ105, the F<sup>'</sup> Cm<sup>R</sup> construct (1,2). For use in preparing uracil-containing M13 templates (3,4).

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### Please Note the Following:

- Streak out the strain on LB agar containing chloramphenicol (15  $\mu$ g/ml) to ensure that you start with an F<sup>'</sup> host.
- Do not include chloramphenicol in liquid media: the strain does not grow well.
- CJ236 is non-suppressing. Do not use M13 vectors that carry amber mutations (mp8, 9 and some mp10 and 11).
- Store plates at 4°C in the dark.

### Preparation of single-stranded uracil-containing template:

- Transform phagemid vector into CJ236 (expect low transformation efficiency with RbCl or CaCl<sub>2</sub> methods).
- Inoculate 50 ml LB (no amp), containing 0.25  $\mu$ g/ml uridine, with a fresh colony. Grow at 37°C with vigorous aeration until slightly turbid (< 10 Klett units).
- Add M13K07 Helper Phage (NEB #N0315S) to a final concentration of 1x10<sup>8</sup> pfu/ml. Continue vigorous aeration for 60–90 minutes.
- Add kanamycin to a final concentration of 70  $\mu$ g/ml. Grow overnight (14–18 hours) with vigorous aeration.

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- Spin culture at 8,000 rpm for 10 minutes. Transfer supernatant to a new tube and spin again.
- Pipet upper 90% of supernatant into a new tube. Add 0.2 volume 2.5 M NaCl/20% PEG 8000. Let sit at 4°C for 60 minutes or overnight.
- Recover phage by centrifugation at 8,000 rpm for 10 minutes. Discard supernatant.
- Resuspend pellet in 1.6 ml TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). Divide into 2 microfuge tubes.
- Spin in microcentrifuge for 5 minutes to pellet any remaining cells. Transfer supernatants to new tubes.
- Add 200  $\mu$ l PEG/NaCl to each. Let sit at room temperature for 5 minutes. Spin in microcentrifuge for 5 minutes.
- Decant supernatant. Spin briefly. Remove last traces of supernatant with pipetman.
- Resuspend each pellet in 300  $\mu$ l TE. Extract with phenol (let sit 15 minutes before spinning), then phenol/chloroform (twice), then chloroform. Add 30  $\mu$ l 2.5 M NaOAc, pH 4.8 and alcohol precipitate.

- Suspend dried pellets in 25–50  $\mu$ l TE. For phagemid with the pUC replication origin, the yield should be > 50  $\mu$ g single-stranded phagemid. For lower copy number vectors, the bulk of the single-stranded DNA will be helper phage at this point.

**Notes:** Storage at -70°C is recommended for periods longer than 30 days. Avoid repeated freeze/thaw cycles.

### References:

- Joyce, C. and Grindley, N. (1984) *J. Bact.* 158, 636–643.
- Raleigh, E. A., Lech, K. and Brent, R. (1989) in Current Protocols in *Molecular Biology eds.* Ausubel, F. M. et al. Publishing Associates and Wiley Interscience; New York. Unit 1.4.
- Kunkel, T. A., Bebenek, K. and McClary J. (1991) *Methods Enzymol.* 204, 125–139.
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