invitrogen™
by life technologies™

One Shot® *ccd*B Survival™ 2 T1^R Chemically Competent Cells

Cat. no. A10460 **Size** 10 reactions **Store at –85°C to –68°C**

Doc. Part no. 100003536 **Pub. no.** MAN0000761 **Rev.** 2.0

Description

The One Shot® *ccd*B Survival™ 2 T1^R strain is resistant to the toxic effects of the *ccd*B gene (Bernard and Couturier, 1992; Bernard et al., 1993) and it may be used to propagate and maintain vectors containing the *ccd*B gene (e.g. Gateway® Technology vectors). The transformation efficiency of One Shot® *ccd*B Survival™ 2 T1^R chemically competent cells is greater than 1×10^9 cfu/µg pUC19 Control DNA.

Component	Amount
*ccd*B Survival™ 2 T1^R Competent Cells	11 × 50 µL
pUC19 Control DNA (10 pg/µL)	50 µL
S.O.C. Medium	6 mL

Genotype

F^- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*Δ139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G *fhuA::IS2*

Certificate of Analysis

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For research use only. Not for use in diagnostic procedures.

General Guidelines

- Handle competent cells gently; they are highly sensitive to changes in temperature and mechanical lysis caused by pipetting.
- Thaw the competent cells on ice, and transform them immediately. Add DNA to tube and mix contents by gentle swirling or tapping. *Do not mix cells by pipetting.*

Cloning Unstable Inserts

When cloning unstable inserts such as lentiviral DNA containing direct repeats (e.g. ViraPower™ Lentiviral Expression Kits), use the following modifications to reduce the chance of recombination between direct repeats:

- Select and culture transformants at 25ºC–30ºC.
- **Do not use** "rich" bacterial medias, because they tend to give rise to a greater number of unwanted recombinants.
- If your plasmid confers chloramphenicol resistance, select and culture transformants using LB medium containing 15–30 μg/mL chloramphenicol *in addition to* the antibiotic appropriate for selection of your plasmid.

Transforming Competent Cells

Before starting the transformation procedure:

- Equilibrate a water bath to 42°C.
- Warm the vial of S.O.C. Medium (supplied with the kit) and LB Medium (if needed) to room temperature.
- Warm the selective plates in a 37°C incubator for 30 minutes (use 1 or 2 plates for each transformation). If you are including the pUC19 control, warm one LB agar plate containing 100 μg/mL ampicillin.

Transformation Procedure

Use this procedure to transform One Shot® *ccd*B Survival™ 2 T1^R chemically competent cells. Include the pUC19 control plasmid DNA to verify the transformation efficiency. *Do not* use these cells for electroporation.

1. Thaw one vial of One Shot® cells on ice for each transformation.
2. Add 1–5 µL of DNA (10 pg to 100 ng) into a vial of One Shot® cells and mix gently. *Do not mix by pipetting up and down.* For the pUC19 control, add 1 µL (10 pg) of DNA into a separate vial of One Shot® cells and mix gently.
3. Incubate the vials on ice for 30 minutes.
4. Heat-shock the cells for 30 seconds at 42°C without shaking.
5. Remove the vials from the 42°C bath and place them on ice for 2 minutes.
6. Add 250 µL of pre-warmed S.O.C. Medium to each vial.
7. Cap the vials tightly and shake horizontally at 37°C for 1 hour at 225 rpm in a shaking incubator.*
8. Spread 25–100 µL from each transformation on a pre-warmed selective plate. Plate two different volumes to ensure that at least one plate will have well-spaced colonies. For the pUC19 control, dilute the transformation mix 1:10 into LB Medium and plate 25–100 µL.
9. Store the remaining transformation mix at 4°C. Additional cells may be plated out the next day, if desired.
10. Incubate plates overnight at 37°C. *

* **Note:** When cloning unstable inserts, incubate the cells at 25ºC–30ºC.

Calculating Transformation Efficiency

Use the following formula to calculate the transformation efficiency as the number of transformants (in cfu) per µg of plasmid DNA.

$$\frac{\text{\# of colonies}}{\text{pg pUC19 DNA}} \times \frac{10^6\ \text{pg}}{\mu\text{g}} \times \frac{\text{volume of transformants}}{\text{X } \mu\text{L plated}} \times \begin{matrix}\text{dilution}\\ \text{factor}\end{matrix}$$

For example, if transformation of 10 pg of pUC19 DNA yields 50 colonies when 25 µL of a 1:10 dilution is plated, then the transformation efficiency is:

$$\frac{\text{50 colonies}}{\text{10 pg DNA}} \times \frac{10^6\ \text{pg}}{\mu\text{g}} \times \frac{300\ \mu\text{L}}{25\ \mu\text{L plated}} \times 10 = 6 \times 10^8$$

References

Bernard, P., Couturier, M., "Cell killing by the F plasmid CcdB protein involves poisoning of DNA-topoisomerase II complexes". *J. Mol. Biol.* (1992) Aug 5; 226(3):735-45.

Bernard, P., Kezdy, K.E., Van Melderen, L., Steyaert, J., Wyns, L., Pato, M.L. , *et al.* (1993) The F plasmid CcdB protein induces efficient ATP-dependent DNA cleavage by gyrase. *J Mol Biol* 234: 534-541.

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