

ChargeSwitch® Plasmid Mini Kit

Cat. no. CS10100

Publication Part no. 25-0811

MAN0008315

Rev. Date 1.0

Follow the steps below to purify up to 20 μL of plasmid DNA from 1–5 mL of fresh overnight cultures grown in LB broth. Use of a richer media may give higher yields. For more detailed protocols and additional information, refer to the kit manual.

1. Before Starting

- 1. For a new kit, mix the RNase A provided in the kit with the Resuspension Buffer (R4).
- 2. Chill the Precipitation Buffer (N5) to 4°C.
- 3. Vortex the ChargeSwitch® Magnetic Beads to resuspend.
- 4. If necessary, warm the Lysis Buffer (L9) to dissolve any precipitate.

2. Preparing the Sample

- 1. In a microcentrifuge tube, pellet cells from 1–5 mL of overnight culture.
- 2. Add 300 μL of Resuspension Buffer, premixed with RNase A as above.
- 3. Add 300 μL of Lysis Buffer (L9), and mix by gentle inversion.
- 4. Incubate at room temperature for 2–5 minutes.
- 5. Add 300 μL of chilled Precipitation Buffer (N5), and mix gently until a white precipitate is formed.
- 6. Centrifuge for 10 minutes at maximum speed.

3. Binding the DNA

- 1. Transfer the supernatant from step 6 above to a new tube containing 40 μL of ChargeSwitch® Magnetic Beads and 90 μL of ETRR (D1).
- 2. Incubate at room temperature for 1 minute, then place the tube in the MagnaRack™ for 1 minute.
- 3. Remove and discard the supernatant, and then remove the tube from the magnet.

4. Washing the Beads

- 1. Add 1 mL of Wash Buffer (W11) to the tube, and gently pipet up and down to mix.
- 2. Place the tube in the MagnaRack™ for 1 minute.
- 3. Remove and discard the supernatant, then remove the tube from the magnet.
- 4. Repeat wash steps 1–3 using 1 mL of Wash Buffer (W12), then proceed to eluting the DNA.

5. Eluting the DNA

- 1. Add 50–150 μL of Elution Buffer (E5) to the tube, and gently pipet up and down to resuspend the beads.
- 2. Incubate at room temperature for 1 minute.
- 3. Place the tube in the MagnaRack™ for 1 minute, or until the beads form a tight pellet.
- 4. Transfer the eluate containing the purified DNA to a new tube.

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7 May 2013

