QUICK REFERENCE

ChargeSwitch® Plasmid Mini Kit

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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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