Quick Reference Card

ChargeSwitch®-Pro Plasmid Miniprep Kit

Catalog nos. CS30050 and CS30250

Part no. 25-0961 Version A, 23 August 2006

Centrifugation Protocol

Follow the steps below to purify plasmid DNA from 1–5 ml of fresh overnight cultures **using a microcentrifuge**. All steps are performed at room temperature. For more detailed protocols and additional information, refer to the kit manual.

1. Before Starting

- ❑ 1.1 For a new kit, add the RNase A provided in the kit to the Resuspension Buffer and mix.
- ❑ 1.2 If necessary, warm the Lysis Buffer to 37°C to dissolve any precipitate.

2. Preparing the Sample

- ❑ 2.1 Pellet cells from 1–5 ml of overnight culture.
- ❑ 2.2 Resuspend in 250 µl of Resuspension Buffer premixed with RNase A. No cell clumps should remain.
- ❑ 2.3 Add 250 µl of Lysis Buffer. Mix well by gentle inversion. Do not vortex.
- ❑ 2.4 Incubate at room temperature for 2–5 minutes. Do not incubate more than 5 minutes.
- ❑ 2.5 Add 250 µl of Precipitation Buffer, and mix well until a white precipitate is formed.
- ❑ 2.6 Centrifuge for 10 minutes at maximum speed to pellet the debris.

3. Binding the DNA

- ❑ 3.1 Carefully transfer the supernatant from Step 2.6 above onto the ChargeSwitch®-Pro MiniPrep Colum inserted in a Collection Tube.
- ❑ 3.2 Centrifuge the column/tube at maximum speed for 30–60 seconds.
- ❑ 3.3 Remove the column from the tube and discard the flow-through. Re-insert the column in the same tube.

4. Washing the Column

- ❑ 4.1 Add 750 µl of Wash Buffer 1 to the column.
- ❑ 4.2 Centrifuge the column/tube at maximum speed for 30–60 seconds.
- ❑ 4.3 Remove the the column from the tube and discard the flow-through. Re-insert the column in the tube.
- ❑ 4.4 Add 250 µl of Wash Buffer 2 to the column.
- ❑ 4.5 Centrifuge the column/tube at maximum speed for 30–60 seconds.
- ❑ 4.6 Remove the column from the tube. Discard the flow-through **and** the Collection Tube.

5. Eluting the DNA

- ❑ 5.1 Insert the column into an Elution Tube (provided in the kit).
- ❑ 5.2 Add 50–100 µl of Elution Buffer onto the column.
- ❑ 5.3 Centrifuge the column/tube at maximum speed for 30-60 seconds.
- ❑ 5.4 **Optional:** Remove the Elution Tube and transfer the eluate back onto the same column. Re-insert the column in the tube and centrifuge at maximum speed for 30–60 seconds. **This step is recommended for maximum recovery.**
- ❑ 5.5 The eluate contains your purified plasmid DNA.

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Vacuum Protocol

Follow the steps below to purify plasmid DNA from 1–5 ml of fresh overnight cultures **using a vacuum manifold**. All steps are performed at room temperature. For more detailed protocols and additional information, refer to the kit manual.

1. Before Starting

- ❑ 1.1 For a new kit, add the RNase A provided in the kit to the Resuspension Buffer and mix.
- ❑ 1.2 If necessary, warm the Lysis Buffer to 37°C to dissolve any precipitate.

2. Preparing the Sample

- ❑ 2.1 Pellet cells from 1–5 ml of overnight culture.
- ❑ 2.2 Resuspend in 250 µl of Resuspension Buffer premixed with RNase A. No cell clumps should remain.
- ❑ 2.3 Add 250 µl of Lysis Buffer, and mix well by gentle inversion. Do not vortex.
- ❑ 2.4 Incubate at room temperature for 2–5 minutes. Do not incubate more than 5 minutes.
- ❑ 2.5 Add 250 µl of Precipitation Buffer, and mix well until a white precipitate is formed.
- ❑ 2.6 Centrifuge for 10 minutes at maximum speed to pellet the debris.

3. Binding the DNA

- ❑ 3.1 Insert the ChargeSwitch®-Pro Miniprep Column into the luer extension of a vacuum manifold.
- ❑ 3.2 Carefully transfer the supernatant from Step 2.6 above onto the column.
- ❑ 3.3 Apply vacuum until the liquid has passed through the column.

4. Washing the Column

- ❑ 4.1 Add 750 µl of Wash Buffer 1 to the column.
- ❑ 4.2 Apply vacuum until the liquid has passed through the column.
- ❑ 4.3 Add 250 µl of Wash Buffer 2 to the column.
- ❑ 4.4 Apply vacuum until the liquid has passed through the column.
- ❑ 4.5 Remove the column from the manifold and insert it into a Collection Tube.
- ❑ 4.6 Centrifuge the column/tube at maximum speed for 30–60 seconds.
- ❑ 4.7 Remove the column from the tube and discard the Collection Tube.

5. Eluting the DNA

- ❑ 5.1 Insert the column into an Elution Tube (provided in the kit).
- ❑ 5.2 Add 50–100 µl of Elution Buffer onto the column.
- ❑ 5.3 Centrifuge the column/tube at maximum speed for 30-60 seconds.
- ❑ 5.4 **Optional:** Remove the Elution Tube and transfer the eluate back onto the **same** column. Re-insert the column in the tube and centrifuge at maximum speed for 30–60 seconds. **This step is recommended for maximum recovery.**
- ❑ 5.5 The eluate contains your purified plasmid DNA.

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