QUICK REFERENCE CARD

ChargeSwitch®-Pro Filter Plasmid Midiprep and Maxiprep Kit

Catalog nos. CS31104, CS31106, and CS31107 **Rev. date: 20 March 2009**; Part no. 100003538; MAN0001638

Centrifugation Protocol

Follow the steps below to purify plasmid DNA from fresh overnight cultures **using a swinging bucket centrifuge**. All steps are performed at room temperature. For more detailed protocols, refer to the manual included with the kit, or go to www.invitrogen.com.

Before Starting

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

Preparing the Sample

❑ 1 Pellet cells from overnight culture.
- **Midiprep**: ≤25 ml
- **Maxiprep**: ≤100 ml

❑ 2 Resuspend cells in Resuspension Buffer premixed with RNase A. No cell clumps should remain.
- **Midiprep**: 5 ml
- **Maxiprep**: 7 ml

❑ 3 Add Lysis Buffer. Mix well by gentle inversion 10 times. **Do not** vortex.
- **Midiprep**: 5 ml
- **Maxiprep**: 7 ml

❑ 4 Incubate at room temperature for 5 minutes. **Do not** incubate for more than 5 minutes.

❑ 5 Add Precipitation Buffer, and mix well until white precipitate forms.
- **Midiprep**: 5 ml
- **Maxiprep**: 7 ml

Clearing/Binding the DNA

❑ 1 Transfer the lysate from Step 5 (above) onto the ChargeSwitch®-Pro Filter Column inserted in a 50 ml conical tube.

❑ 2 Incubate for 2–3 minutes to allow the precipitate to float to the surface.

❑ 3 Centrifuge the column at >2,250 × g for 2–3 minutes.

❑ 4 Gently remove the column from the tube and discard the flow-through.

❑ 5 Remove and discard the inner Lysate Clarification Column, then re-insert the column in the same tube.

Washing the Column

❑ 1 Add 15 ml of Wash Buffer 1 to the column.

❑ 2 Centrifuge the column at >2,250 × g for 1 minute.

❑ 3 Remove the column from the tube and discard the flow-through. Re-insert the column in the tube.

❑ 4 Add 15 ml of Wash Buffer 2 to the column.

❑ 5 Centrifuge the column at >2,250 × g for 1 minute.

❑ 6 Remove the column from the tube. Discard the flow-through **and** 50 ml conical tube.

Eluting the DNA

❑ 1 Insert the column into a clean 50 ml conical tube.

❑ 2 Add Elution Buffer onto the column.
- **Midiprep**: 0.5–1 ml
- **Maxiprep**: 1–2 ml

❑ 3 Centrifuge the column at >2,250 × g for 1 minute.

❑ 4 Remove the column from the tube.

❑ 5 Transfer the eluate from the tube back onto the same column and replace it in the tube.

❑ 6 Centrifuge the column at >2,250 × g for 1 minute. The eluate contains the purified DNA.

❑ 7 Discard the used column.

❑ 8 Store DNA at 4°C or –20°C.

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EveryPrep™ Universal Vacuum Manifold Protocol

Follow the steps below to purify plasmid DNA from fresh overnight cultures **using the vacuum assisted elution protocol for the EveryPrep™ Universal Vacuum Manifold** (Invitrogen Cat. no. K211101). All steps are performed at room temperature. For more detailed protocols, refer to the manual included with the kit, or go to www.invitrogen.com.

Before Starting

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

Preparing the Sample

❑ 1 Pellet cells from overnight culture.
- **Midiprep: ≤25 ml** • **Maxiprep: ≤100 ml**

❑ 2 Resuspend cells in Resuspension Buffer premixed with RNase A. No cell clumps should remain.
- **Midiprep: 5 ml** • **Maxiprep: 7 ml**

❑ 3 Add Lysis Buffer. Mix well by gentle inversion 10 times. **Do not** vortex.
- **Midiprep: 5 ml** • **Maxiprep: 7 ml**

❑ 4 Incubate at room temperature for 5 minutes. **Do not** incubate for more than 5 minutes.

❑ 5 Add Precipitation Buffer, and mix well until white precipitate forms.
- **Midiprep: 5 ml** • **Maxiprep: 7 ml**

Preparing the Manifold

❑ 1 Place the Waste Tray, and Waste Cover, in the Binding Chamber.

❑ 2 Place the Midi/Maxi Top Plate over the Binding Chamber and firmly insert ChargeSwitch®-Pro Filter Columns.

❑ 3 Block remaining holes with stoppers.

Clearing/Binding the DNA

❑ 1 Transfer the supernatant from Step 5 (above) onto the column.

❑ 2 Incubate for 2–3 minutes to allow the precipitate to float to the surface.

❑ 3 Apply vacuum until the liquid has passed through the column. Release the vacuum.

❑ 4 Carefully remove the inner Lysate Clarification Column and discard.

Washing the Column

❑ 1 Add 15 ml of Wash Buffer 1 to the column.

❑ 2 Apply vacuum until the liquid has passed through the column. Release vacuum.

❑ 3 Add 15 ml of Wash Buffer 2 to the column.

❑ 4 Apply vacuum until the liquid has passed through the column. Release vacuum.

Eluting the DNA

❑ 1 Place Elution Rack with clean 2 ml elution tubes at the appropriate positions in the Elution Chamber.

❑ 2 Add Elution Buffer onto the column.
- **Midiprep: 0.5–1 ml** • **Maxiprep: 1–1.5 ml**

❑ 3 Apply vacuum until the liquid has passed through the column.

❑ 4 Release the vacuum.

❑ 5 Transfer the eluate from the tube back onto the same column.

❑ 6 Apply vacuum until the liquid has passed through the column. The eluate contains the purified DNA.

❑ 7 Discard the used column.

❑ 8 Store DNA at 4°C or –20°C.

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