Maxwell® RSC Enviro Total Nucleic Acid Kit

Instructions for Use of Product AS1831.

Quick Protocol

Standard Protocol for Capture, Concentration and Clean-Up

Direct Capture and Concentration

1. Add 40ml of pasteurized wastewater into a 50ml conical tube.
2. Add 0.5ml of Protease Solution. Mix by inversion and incubate for 30 minutes at ambient temperature.
3. Clarify sample by centrifuging at 3,000 × *g* for 10 minutes.
4. Carefully decant 20ml of the supernatant into each of two new 50ml conical tubes.
5. To each tube containing 20ml of the clarified supernatant, add 6ml of Binding Buffer 1 (BBD) followed by 0.5ml of Binding Buffer 2 (BBE).
6. Mix well by inversion.
7. Add 24ml of isopropanol to each tube. Mix well by inversion.
8. Attach a Reservoir Extension Funnel to the PureYield™ Binding Column, then connect the column to the vacuum manifold.
9. Pour the mixture from each tube from Step 7 into the Reservoir Extension Funnel on the PureYield™ Binding Column.
10. Turn on the pump and apply vacuum to capture total nucleic acid on the column.
11. Add 5ml of Column Wash 1 (CWE) and apply a vacuum to pull the liquid through the PureYield™ Binding Column.
12. Add 20ml of Column Wash 2 (RWA) and apply a vacuum to pull the liquid through the PureYield™ Binding Column.
13. Continue the vacuum for an additional 30 seconds after all liquid has passed through the membrane.
14. Release the vacuum and remove the column from the vacuum manifold.
15. Assemble the elution device by placing a 1.5ml microcentrifuge tube into the base of the Eluator™ Vacuum Elution Device.
16. Place the Eluator™ Device assembly onto a vacuum manifold.
17. Add 250µl of preheated (60°C) Nuclease-Free Water to the PureYield™ Binding Column. Apply maximum vacuum until all liquid has passed through the column.
18. Repeat the elution by adding another 250µl of preheated Nuclease-Free Water to the PureYield™ Binding Column.

(continued)

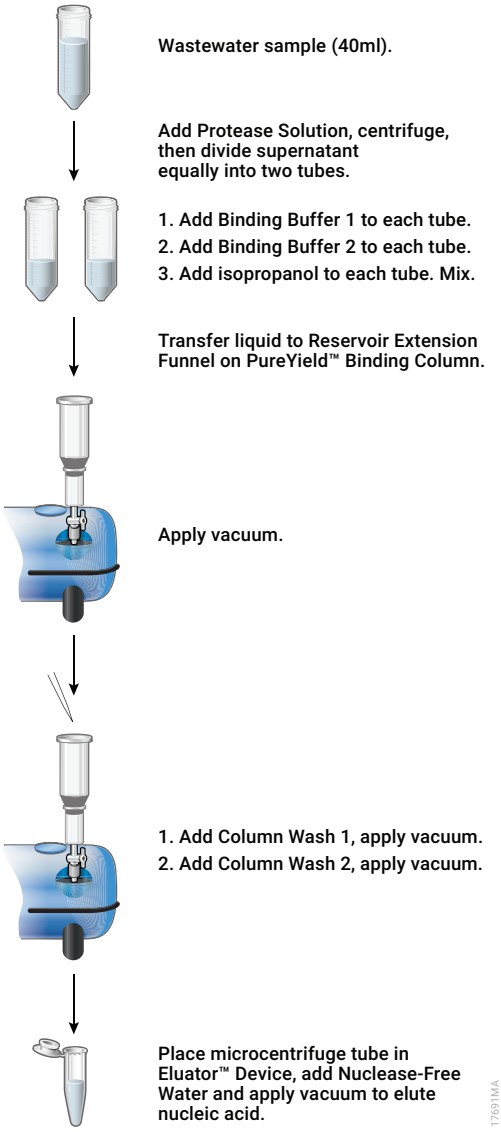

Figure 1. Schematic showing direct capture of nucleic acid from wastewater using a PureYield™ Binding Column.

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Total Nucleic Acid Extraction and Clean-Up on Maxwell® Instrument

Cartridge Preparation

1. Place the cartridge to be used in the Deck Tray(s) with well #1 (the largest well) facing away from the elution tube.
2. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.
3. Place a plunger in well #8 of each cartridge. Well #8 is the well closest to the elution tube.

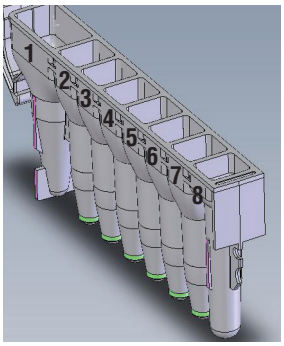

Figure 2. Maxwell® RSC Cartridge. Sample concentrate is added to well #1, plunger added to well #8.

4. Place an empty elution tube in the elution tube position for each cartridge. Add 80µl of Nuclease-Free Water to the bottom of each elution tube.
5. Add 150µl of Binding Buffer 1 and 50µl of Binding Buffer 2 to the 0.5ml of liquid eluted in Step 18.
6. Load the entire volume of mixture to well #1 (the largest well).

Maxwell® Instrument Run

Follow the instrument run instructions in the *Maxwell® RSC Enviro Total Nucleic Acid Kit Technical Manual*, #TM663.

Additional protocol information in Technical Manual #TM663, available online at: **www.promega.com**

