Promega

Wizard® Enviro Total Nucleic Acid Kit

Instructions for Use of Product A2991.

Quick Protocol

Standard Protocol for Capture, Concentration and Clean-Up

1. Dispense 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 8 into the Reservoir Extension Funnel on the PureYield™ Binding Column.
10. Turn on the pump and apply vacuum to capture TNA 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 500µl 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 500µl of preheated Nuclease-Free Water to the PureYield™ Binding Column.

(continued)

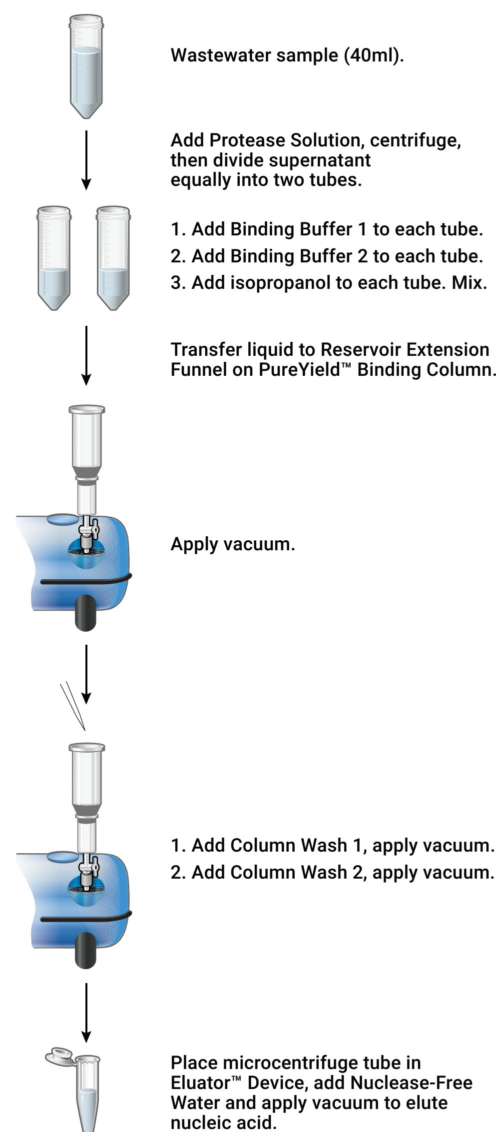

Figure 1. Schematic showing direct capture of total nucleic acid from wastewater using a PureYield™ Binding Column.

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Instructions for Use of Product A2991.

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Quick Protocol

Total Nucleic Acid Extraction and Clean-Up

1. Add 400µl of Binding Buffer 1 (BBD) and 100µl of Binding Buffer 2 (BBE) to 1ml of liquid eluted.
2. Mix well by inversion and divide the contents into two 1.5ml tubes containing 750µl each.
3. Add 750µl of isopropanol to each tube and mix well.
4. Place the PureYield™ Minicolumn into a PureYield™ Collection Tube. Pass the entire volume of the mixture through the column, 750µl at a time, using a microcentrifuge set at 10,000rpm for 1 minute.
5. Add 300µl of Column Wash 1 (CWE) and pull through the PureYield™ Minicolumn by centrifugation. Discard the flowthrough.
6. Add 500µl of Column Wash 2 (RWA) and pull through the PureYield™ Minicolumn by centrifugation. Repeat wash once for a total of two washes. Discard the flowthrough.
7. Centrifuge for 30 seconds to remove any residual wash solution.
8. Transfer the PureYield™ Minicolumn to a new 1.5ml microcentrifuge tube. Add 20µl of preheated (60°C) Nuclease-Free Water to the column. Let the water soak into the column filter for approximately 1 minute.
9. Centrifuge at 10,000rpm for 1 minute to elute. Repeat elution with another 20µl of preheated Nuclease-Free Water, for a total of 40µl.
10. Store sample at or below –20°C until further analysis. Total nucleic acid purified using this method can be used directly in RT-qPCR.

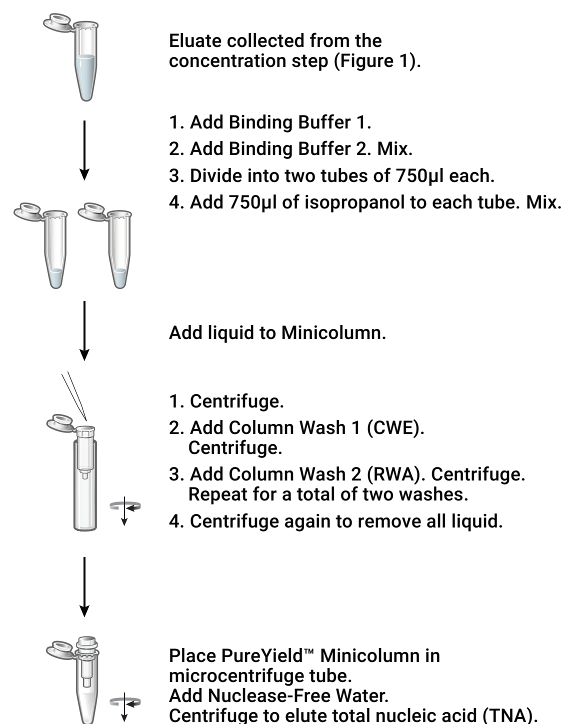

Figure 2. Schematic showing clean-up and concentration of TNA using a PureYield™ Minicolumn.

Additional protocol information in Technical Manual #TM662, available online at: **www.promega.com**

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