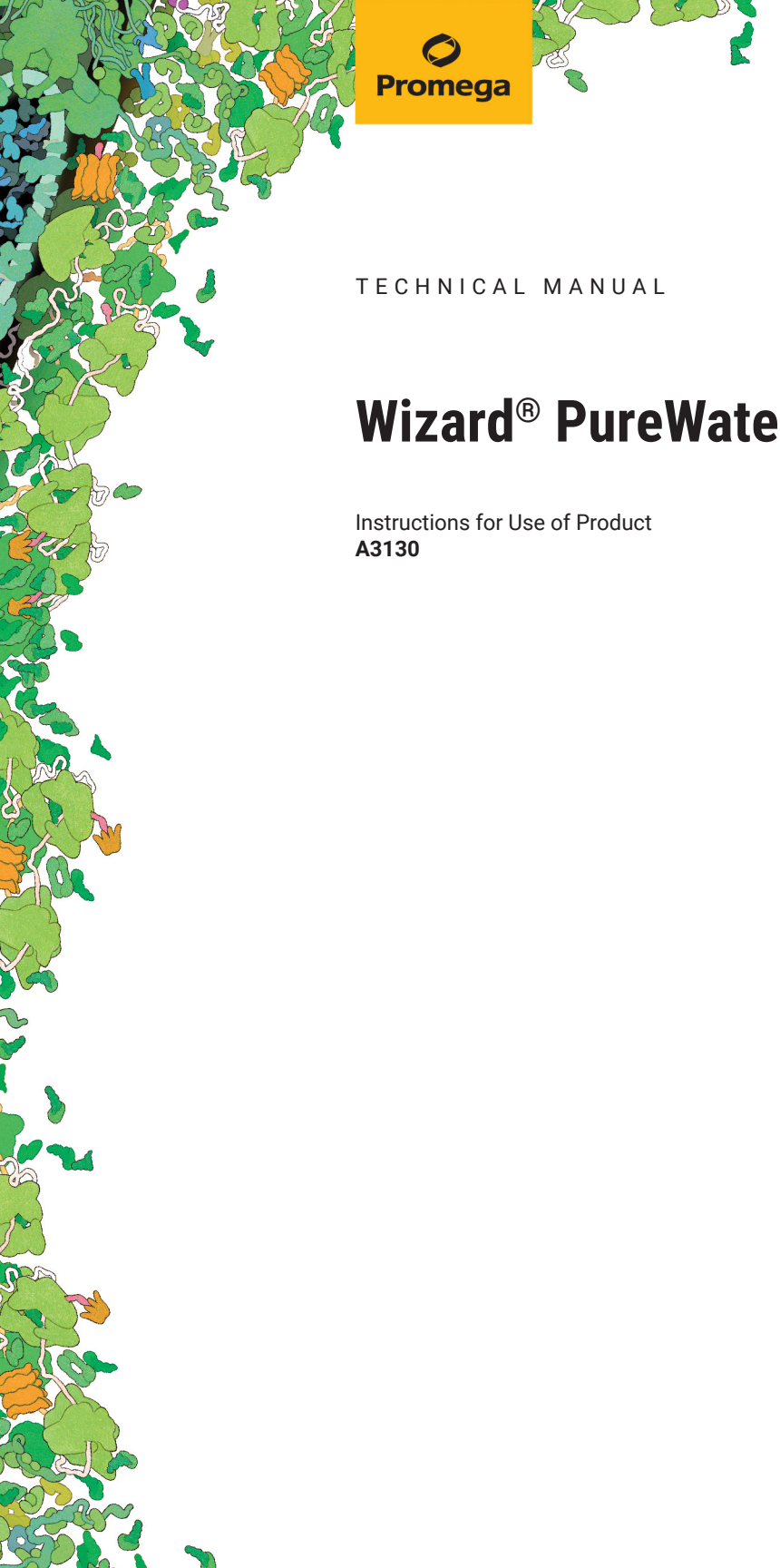

TECHNICAL MANUAL

Wizard® PureWater Kit

Instructions for Use of Product
A3130

11/24
TM729

Wizard® Purewater Kit

All technical literature is available at: www.promega.com/protocols/
Visit the website to verify that you are using the most current version of this Technical Manual.
Email Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The use of molecular tests, and in particular PCR-based assays, continues to expand in water safety testing. The Wizard® PureWater Kit described in this technical manual represents an advancement in the field of molecular water testing, addressing the limitations inherent in traditional manual methods for extracting bacterial DNA from aqueous samples. Current resin, nucleic acid precipitation or membrane column purification approaches are cumbersome and time-consuming, often suffering from inconsistencies due to method variability, which can lead to unreliable results in downstream applications such as PCR, next-generation sequencing (NGS) and microbial source tracking.

This spin-column based method, leveraging the selective binding properties of silica membranes, ensures a high degree of DNA purity and integrity, making it an ideal choice for laboratories aiming for precision and reliability in their molecular analyses. This method uses a short protocol that minimizes the need for specialized laboratory equipment. The purification process is flexible, allowing for the purification of both unconcentrated suspension samples and samples concentrated by filter collection (Figure 1).

The high-quality DNA obtained through this system is optimal for a range of molecular biology techniques, ensuring accurate bacterial pathogen identification and quantification. By streamlining the DNA isolation process, the Wizard® PureWater Kit not only enhances the efficiency of water testing laboratories but also plays a crucial role in safeguarding human health by enabling the rapid and accurate detection of waterborne diseases.

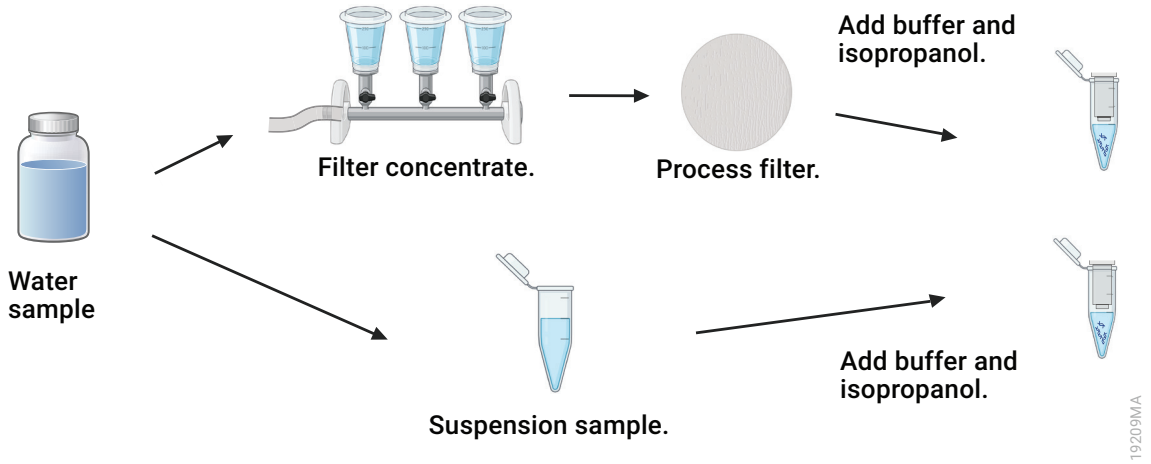

Figure 1. Wizard® PureWater purification workflows. The flexible purification process allows purification of both unconcentrated suspension samples and concentrated samples collected on a filter. (Created using BioRender.com).

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Wizard® PureWater Kit	**50 preps**	**A3130**

Each system contains sufficient reagents for 50 manual nucleic acid isolations from water samples. Includes:

- 20ml Lysis Buffer
- 15ml Column Wash A
- 11.8ml Column Wash B
- 15ml Elution Buffer
- 100ml CTAB Buffer
- 1ml Proteinase K Solution
- 1ml RNase A Solution
- 50 Collection Tubes
- 50 Elution Tubes (1.5ml)
- 1 × 50/pk Reliaprep™ Binding Columns

Storage Conditions: Store the Wizard® PureWater Kit at +15°C to +30°C.

Safety Information: Guanidine hydrochloride (a component of the Lysis Buffer) should be considered harmful and an irritant. Wear gloves and follow standard safety procedures while working with these substances. Refer to the SDS for detailed safety information

3. Before You Begin

Materials to Be Supplied By User

- micropipettes
- 100% isopropanol
- 95% ethanol
- vortex mixer
- heating blocks
- microcentrifuge capable of 14,000 × *g*
- 1.5ml microcentrifuge tubes

Note: Add 10ml of 100% isopropanol to the Column Wash A bottle. Add 20ml of 95% ethanol to the Column Wash B bottle. These volumes are indicated on the Column Wash A and Column Wash B bottles. Make these additions before starting the purification procedure.

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4. Purifying Nucleic Acids from Suspension Samples

Use this protocol if test samples have already been collected and/or resuspended after filter concentration using externally-validated bacterial collection methods (1).

We recommend resuspending bacteria in an isotonic buffer such as 1X PBS (phosphate-buffered saline), instead of nuclease-free water, to minimize osmotic shock that could affect the viability of collected microorganisms.

1. Preheat 100µl of Elution Buffer per sample to 60°C.
2. Add 200µl of each test sample to a 1.5ml microcentrifuge tube.
3. Add 200µl of CTAB and 20µl of Proteinase K to each sample. Pulse vortex to mix.
4. Incubate at 56°C for 10 minutes.
5. Add 300µl of Lysis Buffer to each sample tube and mix by inverting 3–5 times.
6. Add 500µl of 100% isopropanol to each sample. Mix well by inverting 3–5 times.
7. Place ReliaPrep™ Binding Column into a Collection Tube. Pipet 700µl of sample into ReliaPrep™ Binding Column.
8. Centrifuge sample into a Collection Tube at 14,000 × *g* for 1 minute. Discard the flowthrough liquid as hazardous waste.
9. Repeat Steps 7–8 with the remaining sample using the same ReliaPrep™ Binding Column. Discard the flowthrough liquid as hazardous waste.
10. Remove the Collection Tube containing flowthrough, and discard the flowthrough liquid as hazardous waste.
11. Add 300µl of Column Wash A to each ReliaPrep™ Binding Column.
12. Centrifuge sample into a Collection Tube at 14,000 × *g* for 1 minute. Discard the flowthrough liquid as hazardous waste.
13. Add 300µl of Column Wash B to each ReliaPrep™ Binding Column.
14. Centrifuge sample into Collection Tube at 14,000 × *g* for 1 minute. Discard the flowthrough liquid as hazardous waste.
15. Repeat Steps 13–14.
16. Centrifuge at 14,000 × *g* for 30 seconds to remove any residual wash solution.
17. Transfer the ReliaPrep™ Binding Column to a new 1.5ml Elution Tube and add 40µl of preheated (60°C) Elution Buffer to the column. Let the buffer soak into the column filter for approximately 1 minute.
18. Centrifuge at 14,000 × *g* for 1 minute to elute. Repeat elution with another 40µl of preheated Elution Buffer, for a total volume of 80µl in the 1.5ml Elution Tube
19. Use purified nucleic acid in PCR or other desired molecular analyses. Alternatively, store samples at –30°C to –10°C for later analysis.

5. Filtering, Concentrating and Purifying Nucleic Acid from Bulk Water Samples

Use this protocol to filter concentrate bacteria from bulk water or liquid samples. Suggested filter material and filter apparatus setups are listed below, but any validated filter collection method can be used.

This protocol uses 25mm filters. Please contact Promega Technical Services (**techserv@promega.com**) for prototype instructions using 47mm filters.

Materials to Be Supplied By User

- filter concentration apparatus, including:
  - vacuum manifold (e.g., Cytiva Cat.# 4889);
  - standard adapter (e.g., Cytiva Cat.# 4892);
  - 25mm filter funnel (e.g., Cole Parmer Cat.# EW-35200-55);
  - rubber stoppers (VWR Cat.# 59581-367)
- 0.2µm polycarbonate membrane filters 25mm diameter (e.g., Millipore Cat.# GTTP02500) or 0.2µm polycarbonate membrane filters 47mm diameter (e.g., Millipore Cat.# GTTP-04700)
- Welch® vacuum pump (for North America, Model 2522B-01, Cat.# A6270; for Europe, Model 2522C-02, Cat.# A6722) or other vacuum source
- 1X PBS (phosphate-buffered saline)
- 100% isopropanol
- 95% ethanol
- 2ml lo-bind microcentrifuge tubes (e.g., Eppendorf Cat.# 0030108426)
- sterile scalpel
- micropipettes
- sterile petri dishes
- heating blocks
- vortex mixer

5.A. Filter Collection and Concentration

1. Collect desired volume (100ml or greater) of each water sample to be tested.
2. Filter concentrate the sample onto a 0.2µm polycarbonate filter.
3. Aseptically transfer the filter to a sterile petri dish.
4. Fragment the filters into halves or quarters using a sterile scalpel.
5. Aseptically transfer the fragmented filter to a 2ml microcentrifuge tube.
6. Add 700µl of 1X PBS to each tube.

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5.B. Sample Pretreatment and Processing

1. Preheat 100µl of Elution Buffer per sample to 60°C.
2. Centrifuge the 2ml microcentrifuge tubes with filters at 14,000 × *g* for 1 minute.
3. Carefully remove liquid from filters in the tubes, pipetting from center of the tube bottom to avoid removing the pelleted bacteria.
4. Add 700µl of CTAB buffer to the 2ml microcentrifuge tube containing filter. Vortex for 30 seconds.
5. Incubate at 95°C for 5 minutes. Cool at room temperature for 2 minutes. Vortex for 1 minute.
6. Add 20µl of Proteinase K and 10µl of RNase A Solution to each 2ml tube, then vortex to mix.
7. Incubate at 70°C for 10 minutes.
8. Add 300µl of Lysis Buffer to each sample. Mix by inverting 3–5 times.
9. Add 700µl of 100% isopropanol to each sample. Mix well by inverting 3–5 times.
10. Place ReliaPrep™ Binding Column in a Collection Tube. Add 800µl of liquid sample to a ReliaPrep™ Binding Column.
    Note: Do not transfer filters to the binding column.
11. Centrifuge sample into Collection Tube at 14,000 × *g* for 1minute.
12. Repeat Steps 10–11 a total of three times, to load entire sample onto ReliaPrep™ Binding Column. After centrifugation, discard flowthrough.
13. Add 300µl of Column Wash A to each column.
14. Centrifuge sample into Collection Tube at 14,000 × *g* for 1minute. Discard flowthrough.
15. Add 300µl of Column Wash B to each column.
16. Centrifuge sample into Collection Tube at 14,000 × *g* for 1minute. Discard flowthrough.
17. Repeat Steps 15–16.
18. Centrifuge at 14,000 × *g* for 30 seconds to remove any residual wash solution.
19. Transfer the ReliaPrep™ Binding Column to a new 1.5ml Elution Tube and add 40µl of preheated (60°C) Elution Buffer to the column. Let the buffer soak into the column filter for approximately 1 minute.
20. Centrifuge at at 14,000 × *g* for 1 minute to elute. Repeat elution with another 40µl of preheated Elution Buffer, for a total volume of 80µl.
21. Proceed to PCR or other desired molecular analyses of purified nucleic acid. Alternatively, store samples at –30°C to –10°C for later analysis.

6. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. Email: **techserv@promega.com**

Symptom	Causes and Comments
Lower than expected DNA yield	Ensure that isopropanol and ethanol are added to Column Wash A and Column Wash B, respectively, before starting purification procedure. See the Column Wash bottle labels for volumes to add.
	Filter concentrate a larger volume of liquid sample.
	Sample has lower than expected DNA content or has degraded.
Precipitate in CTAB or Lysis Buffer bottle	Precipitate may form at lower temperatures. Resuspend the precipitated solution by warming the bottle at room temperature, and shaking.
Downstream amplification reactions appear inhibited	Ethanol carryover in the eluted DNA sample can inhibit downstream enzymatic reactions. Prior to the final DNA elution step, confirm that the Binding Column is dried by centrifuging at maximum speed for 30 seconds.

7. References

1. 9260 Introduction to Detecting Pathogenic Bacteria In: Standard Methods For the Examination of Water and Wastewater. Standard Methods Committee of the American Public Health Association, American Water Works Association, and Water Environment Federation. Lipps WC, Baxter TE, Braun-Howland E, eds. Washington DC. APHA Press. Accessed 4-September-2024. **doi: 10.2105/SMWW.2882.201**

8. Related Products

Product	Size	Cat.#
GoTaq® *Legionella pneumophila* qPCR Kit	1 each	AM2201
GoTaq® *Legionella spp/pneumophila*/SG1 qPCR Kit	1 each	AM2202
GoTaq® *Legionella pneumophilia* Viability qPCR Kit	1 each	AM2205
GoTaq® *Legionella spp/pneumophila*/SG1 Viability qPCR Kit	1 each	AM2206
Viability PCR Reagent System	1 kit	A8881
Viability PCR Reagent System, High Concentration	1 kit	A8883
Maxwell® RSC PureWater Kit	48 preps	AS2110
Wizard® Enviro TNA Kit	1 each	A2991

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