AquaRNA™ Instruction Manual

General Information

Description

AquaRNA™ (formerly AquaStool™, AquaPreserve™) is a multifunctional aqueous reagent for fecal sample stabilization, DNA and RNA extraction and PCR inhibitor removal. It may be used to extract fecal DNA for non-invasive genotyping of transgenic animals or preserve and extract DNA/RNA from human and animal stools for host and gut microbiome research and viral, bacterial, fungal, and parasitic detection. Additionally, AquaRNA may also be used to extract total DNA/RNA from various biospecimens, including cultured cells, microbes, blood, saliva, and animal or plant tissues.

Specification

Product Name	AquaRNA™ Solution
Product #	AR5030M
Size	30 ml AquaRNA™ for 200 mouse pellet extractions
MSDS	Available at www.boltii.com
Storage	Store tightly capped at 22 °C. Vortex to mix well before dispensing.
Note	Order these ancillary reagents with AquaRNA™ for specific applications: • AquaRemove™ (AR1208M) for human fecal DNA/RNA • ProSink™ (RS9015M) for blood, liver, pancreas and spleen DNA/RNA

Terms & Condition

Product Usage: For *In Vitro* Laboratory Research Use Only. NOT to be administered to humans or used for medical diagnosis.

Safety, Handling, and Warnings: Contains guanidine thiocyanate, is harmful if swallowed and causes irritation to skin, eyes and respiratory tract. Do not mix with Bleach.

Warranties and Liabilities: Boltii Diagnostics, Inc. accepts no responsibility and shall not be held liable for any loss, damage, expense, consequential, or accidental damage, including damage to property, person, or premises arising out of the use, the results of use, or the inability to use these products. Boltii Diagnostic, Inc. MAKES NO WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING, WITHOUT LIMITATION, WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE.

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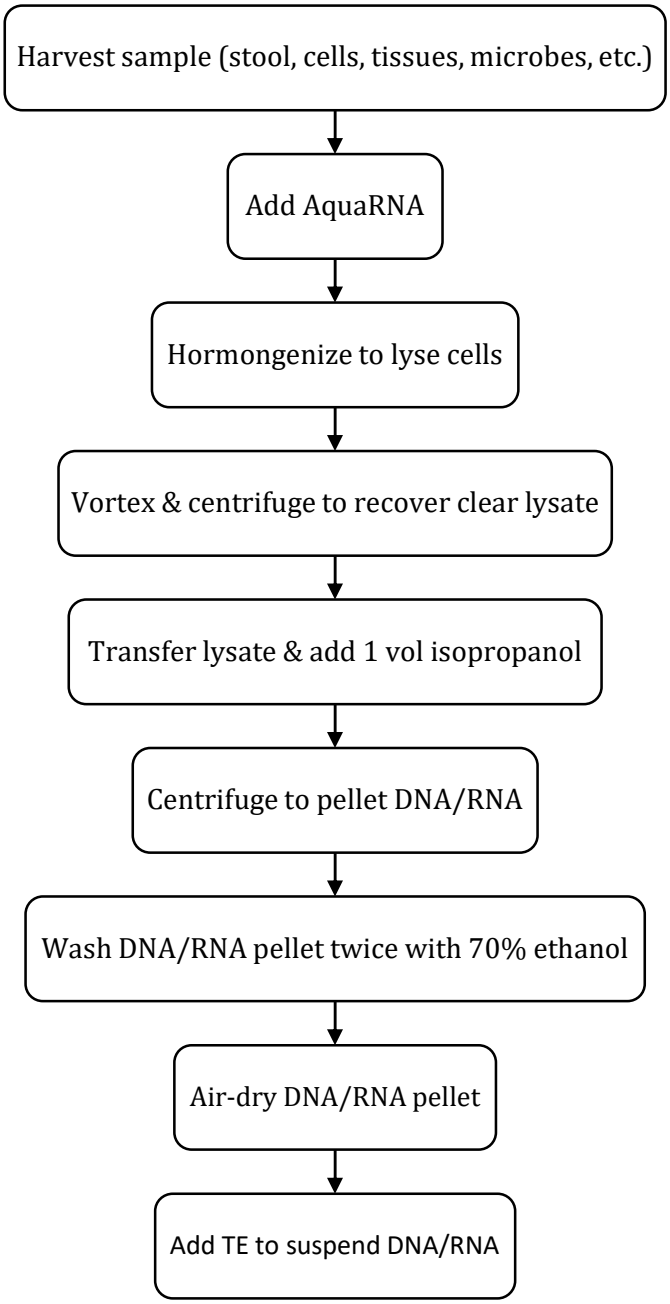

AquaRNA™ Protocol Flowchart

Cultured Cell DNA/RNA Protocol

Extract DNA/RNA from cultured cells (~1–2 million cells).

1. Harvest Cells

- Pellet 1–2 × 10^6 cells at 3,000×g for 5 min.
- Carefully aspirate all medium.

2. Cell Lysis

- Add **100 µL AquaRNA™** to the cell pellet.
- Vortex vigorously for 1 min until no visible clumps remain.
- Incubate at 22 °C for 15 min to lyse cells.

3. Pellet Debris

- Centrifuge at 12,000×g for 5 min.
- Transfer the clear supernatant to a new tube without disturbing the pellet.

4. DNA/RNA Precipitation

- To pellet **mRNA**: Add a quarter volume of isopropanol.
- To pellet **total DNA/RNA**: Add an equal volume of isopropanol.
- Vortex 30 s.
- Centrifuge 12,000×g for 5 min to pellet mRNA or total DNA/RNA.
- Flip tube to discard supernatant.

5. Ethanol Wash

- Rinse the pellet with **70% ethanol** (fill the tube, including cap).
- Discard ethanol and repeat once.
- Tap tube on paper towel to remove residual ethanol.
- Air-dry pellet for 15 min.

6. DNA/RNA Solubilization

- Add **100 µL TE buffer**; pipette and vortex to disperse.
- Incubate at 22 °C for 15 min to solubilize DNA/RNA.
- Spin 5 min and transfer the DNA/RNA-containing supernatant to a new tube.
- Store at -20 °C.

Mouse Fecal DNA Protocol

Extract fecal DNA from mouse fecal pellets for genotyping.

1. Sample Collection

- Place individual mouse in a cage with clean lining.
- Collect pellets in labeled tubes. Air-dry at 37 °C for 24 h if long-term storage is desired.

2. Lysis

- Add ~25 mg white sand and **150 µL AquaRNA™** in a 1.5-mL tube.
- Add a mouse pellet.
- Incubate 22–65 °C for 15–30 min to soften the pellet.
- Vortex/bead-beat sample thoroughly.

Proceed through Steps 3–6 as described in the Cultured Cell DNA/RNA Protocol.

7. PCR genotyping

- Centrifuge the frozen DNA solution to pellet any insoluble (contains PCR inhibitor).
- Use 1 µL clear DNA supernatant per 25-µL PCR reaction.

Human Fecal DNA/RNA Protocol

Extract fecal DNA/RNA from stool samples of human and other large animals.

1. Sample Collection

- Add ~1 g stool in 10 mL AquaRNA™ in a 15-mL tube.
- Smash and disperse the stool with a spoon or rod.
- Close the tube and shake vigorously.
- Ship at ambient temperature for DNA; use cold shipping for RNA.
- Store at -80 °C.

2. Lysis

- Thaw the stool sample and shake vigorously.
- Transfer 0.5 mL stool sample to a 1.5-mL tube preloaded with Add ~100 mg white sand.
- Vortex/bead-beat sample thoroughly.
- For better PCR inhibitor removal: Add 250 µL AquaRemove™ (1:1 diluted with isopropanol), vortex and incubate 5–10 min.

Proceed through Steps 3–6 as described in the Cultured Cell DNA/RNA Protocol.

Tissue DNA/RNA Protocol

Extract DNA/RNA from animal or plant tissues.

1. Prepare Sample

- Cut ~2 mm tissue cubes (~25 mg).

2. Lysis

- Add **500 µL AquaRNA™**.
- Homogenize with a pestle-and-tube homogenizer or a multi-channel bead beater.
- After homogenization, add 2-3 drops of isopropanol to quench foaming.
- For plant tissue: Add 250 µL AquaRemove™ (1:1 diluted with isopropanol), vortex and incubate 5–10 min.

Proceed through Steps 4–6 as described in the Cultured Cell DNA/RNA Protocol.

Microbial DNA/RNA Protocol

Extract DNA/RNA from bacteria or yeast.

1. Harvest Cells

- Pellet 1 mL overnight culture at 12,000×g for 1 min.
- Aspirate supernatant.

2. Cell Lysis

- Pre-treat bacteria with **lysozyme**, or yeast with **lyticase**.
- Pellet cells and remove supernatant.
- Add **500 µL AquaRNA™**.
- Incubate **22 °C for 15 min**.
- Vortex vigorously for 1 min.

Proceed through Steps 3–6 as described in the Cultured Cell DNA/RNA Protocol.

Viral DNA or RNA Protocol

Extract DNA or RNA from DNA or RNA vrius.

1. Harvest Virions

- Centrifuge 50 mL infected culture at 12,000×g for 1 min.
- Transfer supernatant to new tube.
- Add 1 volume 20% PEG8000 in 2.5M NaCl to 5 volume viral supernatant.
- Vortex and centrifuge at 12,000g for 10 min to pellet the virions.
- Aspirate to remove PEG supernatant as completely as possible.

2. Virion Lysis

- Add **500 µL AquaRNA™** and vortex to suspend the viral pellet.
- Incubate **on ice for 15 min**.
- Vortex vigorously for 1 min.
- Transfer viral lysate to 1.5-mL tube.

Proceed through Steps 3–6 as described in the Cultured Cell DNA/RNA Protocol.

Saliva DNA/RNA Protocol

Extract DNA/RNA from human saliva.

1. Collection

- Saliva: Collect **500 µL** after cheek rubbing.

2. Lysis

- Mix **500 µL sample + 500 µL AquaRNA™**.
- Incubate **15 min at 22 °C**.
- Vortex/bead-beat sample thoroughly.
- Add 250 µL AquaRemove™ (1:1 diluted with isopropanol) and vortex.

Proceed through Steps 3–6 as described in the Cultured Cell DNA/RNA Protocol.

Whole Blood DNA/RNA Protocol

Extract total DNA/RNA from whole blood.

1. Collection

- Collect anticoagulated blood using standard procedures.

2. Lysis

- Add **250 µL AquaRNA™** to 250 µL fresh or frozen blood (don't thaw blood without AquaRNA if you intend to recover blood RNA).
- Vortex to thaw blood and incubate **15 min at 22 °C**.
- Add **125 µL ProSink** to precipitate proteins.
- Vortex and incubate at 22 °C for >30 min.

Proceed through Steps 3–6 as described in the Cultured Cell DNA/RNA Protocol.

Plasma DNA/RNA Protocol

Extract cell-free plasma or serum DNA/RNA.

1. Collection

- Prepare the plasma (or serum) from fresh whole blood using standard methods.
- Transfer 2x 0.5 ml plasma to 2x 2-mL microfuge tubes.

2. Extraction

- Add **0.5 mL AquaRNA™** to each; vortex and incubate **15 min at 22 °C**.

Proceed through Steps 4–6 as described in the Cultured Cell DNA/RNA Protocol.

Frequently Asked Questions

Please read through these questions carefully. The answers provide additional helpful tips and useful information for the successful use of AquaRNA.

1. How should I store the AquaRNA solution?

AquaRNA may be stored at 22 °C for 12 months. If AquaRNA becomes precipitated when exposed to low temperature, you may incubate it at 37-50 °C for 15-20 min to resolubilize the reagent.

2. Why shouldn't I use Bleach to disinfect AquaRNA preserved fecal specimen?

AquaRNA contains guanidine thiocyanate. It may react with Bleach (sodium hypochlorite) and release toxic gases.

3. How should I air-dry the mouse fecal samples?

Air-dried mouse fecal samples can be stored long term at room temperature for future genotype verification. To air-dry mouse fecal pellets, incubate the fecal pellets in an opened microfuge tube on a dry heat bloc at 37 °C for 24 hours.

4. Why is my DNA/RNA solution showing a strong absorption below A260?

It is likely due to trace amount of guanidine salt contamination. If it interferes with your downstream applications, you may further purify the extracted DNA/RNA with a silica spin column (e.g., a plasmid miniprep column). Simply add an equal volume of 4 M GuHCl and 1M NaOAc (pH unadjusted, ~7.0) to your DNA/RNA solution and load it into the spin column, centrifuge to allow DNA/RNA binding to the silica membrane, wash the column with 0.6 ml 75% ethanol, and elute the DNA/RNA in 50 µL deionized water or TE buffer.

5. My mouse transgene was not amplified well, how may I improve it?

Try the following to improve mouse fecal DNA amplification: (a) after freezing the fecal DNA solution at -20 °C, re-centrifuge it to pellet and remove any insoluble, which may contain PCR inhibitors; (b) reduce the amount of fecal DNA used per PCR reaction (i.e., try using 0.5, 0.25, 0.1 and 0.01 µL extracted fecal DNA per PCR reaction); (c) increase PCR cycles up to 65; (d) add 1 mM DTT and 0.1 mg/mL BSA to the PCR reaction; (e) use a gel imager to visualize faint amplicon bands; (f) use AquaRemove™ with AquaRNA to purify fecal DNA (see "Human fecal DNA/RNA extraction" for details); and (g) further purify the fecal DNA with a silica spin column as outlined in #4 Question and Answer above.