General Information

Description

AquaRNA™ is a multifunctional aqueous solution for DNA, RNA, and protein extraction. It lyses the cells, inactivates degradative enzymes, and extracts DNA, RNA, and proteins. DNA and RNA are recovered from the cell lysate by isopropanol precipitation, while proteins remain soluble in the isopropanol solution and can be recovered by acetone precipitation. AquaRNA™ enables concurrent isolation of DNA, RNA, and proteins from the same specimen for analyses without using different DNA, RNA, and protein extraction kits.

Specification

Product Name	AquaRNA™ Kit
Product #	5001, 5030
Size	5001: 1 ml; 5030: 30 ml
Kit Contents	5001: 1 ml AquaRNA Solution, Instruction Manual 5030: 30 ml AquaRNA Solution, Instruction Manual
MSDS	Available at www.aquaplasmid.com
Storage	Store tightly capped at 4 °C. Vortex to mix well before dispensing.
Note	In addition to AquaRNA, please order ProSink (# 9015) for protein removal when extracting RNA from blood, liver, spleen, and other animal tissues; and ProMelt (# 1115) for protein solubilization when extracting proteins.

Terms & Condition

Product Usage: For In Vitro Laboratory Research Use Only. NOT to be administered to humans or used for medical diagnosis.
Limited Product Warranty: We offer a LIMITED PRODUCT WARRANTY to our customers. This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by MultiTarget Pharmaceuticals. We shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.
Product Warning: Contains guanidine thiocyanate. Harmful if swallowed. Causes irritation to skin, eyes, and respiratory tract. Do not mix with Bleach.

Patents, Trademarks & Copyrights

AquaRNA Protocol

This protocol uses 0.5 ml AquaRNA to extract DNA, RNA, and proteins from 5 million cultured cells, or 25 mg of animal tissues, or 50 mg of plant tissues. You may scale up or down the volume of AquaRNA proportionately for different amount of starting materials.

1. Lyse the Cells

For eukaryotic cells: Transfer ~5 million cells in 250 µl of culture medium or trypsin cell dissociation solution to 500 µl of AquaRNA preloaded in a 1.5-ml microfuge tube, vortex to mix well and invert the tube to wet the entire interior of the tube. Centrifuge at 14,000 xg for 5 min to pellet the debris (usually the cells are completely lysed and there is no debris pellet after the centrifugation).

For microbial cells: Centrifuge 1 ml log-phase culture at 14,000 xg for 30 seconds to pellet the cells. Aspirate to discard the medium and suspend the cells in 250 µl of 1 mg/ml lysozyme (not included, use lyticase or equivalent for yeast cells) in TE buffer (pH 8, lysozyme will not be as effective at pH <8) and incubate on ice for >15 minutes, and vortex occasionally. Transfer the cell suspension to 500 µl of AquaRNA preloaded in a 1.5-ml microfuge tube, vortex to lyse the cells (Note: A bead beater may be needed for optimal cell lysis, especially for Gram+ bacteria). Centrifuge at 14,000 xg for 5 min to pellet the debris.

For animal tissues: If a multi-channel bead beater is available, homogenize ~25 mg tissue in 500 µl of AquaRNA with a mix of sixty 1.4-mm and six 2.8-mm ceramic beads. Otherwise, homogenize the plant tissue with a pestle-and-tube homogenizer, move the pestle up and down while vortexing at maximal speed to fully homogenize the tissue. Be careful to avoid excess foaming. After homogenization, add 2-3 drops (20-40 µl) of isopropanol to the sample to reduce foaming, vortex and pour the homogenate into a new microfuge tube. Centrifuge at 14,000 xg for 5 min to pellet the debris.

For plant tissues: If a multi-channel bead beater is available, homogenize ~50 mg tissue in 500 µl of AquaRNA with a mix of sixty 1.4-mm and six 2.8-mm ceramic beads. Otherwise, homogenize the plant tissue with a pestle-and-tube homogenizer, move the pestle up and down while vortexing at maximal speed to fully homogenize the tissue. Be careful to avoid excess foaming. After homogenization, add 2-3 drops (20-40 µl) of isopropanol to the sample to reduce foaming, vortex and pour the homogenate into a new microfuge tube. Centrifuge at 14,000 xg for 5 min to pellet the debris.

2. Extract the Proteins

(Go to Step 3. Extract the DNA and RNA, if you don't extract the proteins.) Transfer 100 µl clear lysate to a microfuge tube. Add 0.8 vol (80 µl) isopropanol, vortex for 60 sec, and centrifuge at 14,000 xg for 5 min to pellet the DNA and RNA. Transfer the protein-containing supernatant (150 µl) to a new 1.6-ml microfuge tube. Add 4 vol (0.6 ml) acetone, vortex for 60 sec, and centrifuge at 14,000xg for 5 min to pellet the proteins. Decant to discard the supernatant, tap the tube on a clean paper towel to remove residual acetone. Immediately add 200 µl ProMelt to the wet protein pellet, pipette and vortex to suspend the pellet. Incubate at 22 °C for 15 min to solubilize the proteins. Vortex and centrifuge at 14,000xg for 5 min to pellet any insoluble material. Transfer the protein solution to a new tube and store at 4 or –20 °C (Some SDS may precipitate out at these temperatures, however, it will not interfere with SDS-PAGE. Or it may be re-solubilized by incubating at 65 °C for 10 min.).

3. Extract the DNA and RNA

For DNA/RNA extraction from low protein content specimens, such bacteria, cultured cells, or plant tissues, add 0.8 vol (240 µl) isopropanol to 1 vol (300 µl) clear lysate. Vortex for 60 sec, and centrifuge at 14,000 xg for 5 min to pellet the DNA/RNA.

For DNA/RNA extraction from protein-rich and RNase-rich specimens, such as liver and spleen tissues, add 1/3 vol (100 µl) ProSink to 1 vol (300 µl) clear lysate. Vortex for 60 sec and centrifuge at 14,000 xg for 5 min to pellet the proteins. Transfer the supernatant (350 µl) to a new tube and add 0.8 vol (280 µl) isopropanol. Vortex for 60 sec and centrifuge at 14,000 xg for 5 min to pellet DNA/RNA.

Decant to discard the supernatant. Gently fill the tube with 50% isopropanol (or 70% ethanol) from a squirt bottle and then decant to discard the alcohol solution. Be sure to rinse the entire interior of the tube, including the inside of the cap. Repeat the alcohol rinse 2-3 times. Tap the tube on a paper towel to remove residual alcohol, leave the tube upside down on the paper towel for 5-10 min to air dry the DNA/RNA pellet. Add 300 µl of nuclease-free water to the pellet, vortex and/or pipette to solubilize the DNA/RNA pellet. Incubate at 22 °C for 5 min, centrifuge at 14,000 xg for 5 min to pellet any insoluble material, transfer the DNA/RNA solution to a new tube and store at –20 °C.

If DNA removal is desired, add 0.2 units of DNase I to 10-20 µl of DNA/RNA solution, and incubate at 22-37 °C for 20-30 min. Then run the digested sample in a 0.8% native agarose gel to confirm that the DNA digestion is complete and the RNA bands are discrete. To inactivate the DNase I, use Ambion's DNase removal reagent or heat-inactivate the DNase I at 65 °C for 15 min.

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?

It may be stored at 4 °C for 12 months. Vortex to mix the reagent well before dispensing.

2. I did not see the 28S and 18S rRNA bands in the gel, why?

The 28S and 18S rRNA bands migrate with the genomic DNA. You may remove the DNA with DNase I digestion and then run the digested sample in a native 0.8% agarose gel at 100-120V for 20-30 min. You only need to run the bromophenol blue dye ~1.5-2 cm into the gel to get a good separation of the sharp RNA bands. Longer run may cause the RNA bands become smear because RNA can adopt different conformations in a native gel.

3. Do I need to add RNase Inhibitor to the final RNA solution?

No. AquaRNA is very effective at inactivating and removing RNases. You could even leave your RNA samples at room temperature for days without noticing any RNA degradation.

4. My RNA was degraded, where was the RNase coming from?

To troubleshooting RNase contamination, you may set up a DNase I digestion. Before adding DNase I, you may divide the sample into two aliquots and add DNase I to one of them. If RNA degradation is seen only in the DNase I treated sample, your DNase I may be contaminated. If RNA is degraded without adding DNase I, the DNA/RNA prep may be contaminated. To prevent RNase contamination of the DNA/RNA prep, you should make sure your gloves or fingers do not touch the inside of the lid and the mouth of the tube when opening and closing the tube containing the DNA/RNA solution.

5. Do I need to use ProMelt and ProSink?

ProMelt (Item # 1115) is an ancillary reagent for solubilizing protein pellet precipitated by acetone. ProMelt is not needed, if you don't intend to recover proteins. ProSink (Item # 9015) is a protein precipitating solution for RNA extraction from blood or other protein-rich animal tissues. ProSink is optional, if you extract DNA and RNA from bacteria, cultured cells, or plant tissues.

6. Can I do RT-PCR without removing the contaminating genomic DNA?

DNA removal may be unnecessary if you design and use a 5' tailed RT primer to make the cDNA and then use a pair of PCR primers, with one of them complementary to the unique tailed region of the RT primer to amplify the cDNA [Hurteau and Spivack. mRNA-specific reverse transcription-polymerase chain reaction from human tissue extracts. Anal Biochem. 2002 Aug 15;307(2):304-15; and Chen, et al. Real-time quantification of microRNAs by stem–loop RT–PCR. Nucleic Acids Research 2005 33(20):e179].