AquaRNA Instruction Manual

General Information

Description

AquaRNA™ is a multifunctional aqueous solution-based reagent for DNA, RNA, and protein extraction. This single solution will lyse the cells, inactivate degradative enzymes, and extract 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 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, User Manual 5030: 30 ml AquaRNA Solution, User Manual
MSDS	Available at www.aquaplasmid.com
Storage	Store tightly capped at 22 °C. Vortex to mix well before dispensing.
Note	In addition to AquaRNA, please order ProSink (# 9030) for protein removal when extracting RNA from blood, liver, and spleen; 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 Cell Protocol

This protocol uses 100 µl of AquaRNA to extract DNA, RNA, and proteins from 0.5-2 million cultured mammalian cells or 0.25 ml log-phase microbial culture. Invert to mix the reagent well before dispensing.

1. Harvest the cells

For eukaryotic cells: Centrifuge at 3,000 xg for 5 min to pellet 0.5-2 million cultured cells in a 1.5-ml microfuge tube, and aspirate to remove the medium *(Note: Centrifuging to pellet the cells is not needed as long as 1 vol of AquaRNA is mixed with 1 vol of cell suspension for DNA/RNA extraction.)*.

For microbial cells: Centrifuge 0.25 ml log-phase culture at 14,000 xg for 1 min to pellet the cells. Aspirate to remove the medium and suspend the cells in 100 µl of 1 mg/ml lysozyme (not included, use lyticase or equivalent for yeast cells) in TE buffer containing 300 mM KCl (*pH 8, lysozyme will not be as effective at pH <8*) and incubate on ice for >15 minutes, and vortex occasionally.

2. Extract the DNA/RNA

Add 100 µl of AquaRNA to the mammalian cell pellet or the lysozyme-digested bacterial suspension. Vortex to mix well and invert the tube to wet the entire interior of the tube (*for Gram+ bacteria, use a bead beater to assist cell lysis*). Optional: Centrifuge at 14,000 xg for 5 min to pellet the cell debris (*Note: Centrifuging to pellet the cell debris is not needed for mammalian cells. Addition of 0.8 vol of isopropanol to the crude lysate in the next step will make the lysis go to completion and increase DNA/RNA yield.*).

3. Pellet the DNA/RNA

Transfer the lysate (~90 µl) to a new 0.5-ml microfuge tube. Add 0.8 vol (~72 µl) of isopropanol and vortex to mix. Centrifuge at 14,000 xg for 5 min to pellet the DNA/RNA. Decant to discard the supernatant (*Note: Proteins remain in the isopropanol supernatant and can be recovered by precipitation in 4 vol of acetone*.). Gently fill the tube with 70% ethanol from a squirt bottle and then decant to discard the ethanol solution. Be sure to rinse the entire interior of the tube, including the inside of the cap. Repeat the ethanol rinse once. Tap the tube on a paper towel to remove residual ethanol, place the tube upside down on the paper towel to air dry the DNA/RNA pellet for 5-10 min. Add 100 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, and transfer the DNA/RNA solution to a new tube.

AquaRNA Tissue Protocol

This protocol uses 0.5 ml of AquaRNA to extract DNA, RNA, and proteins from 25 mg of animal tissues or 50 mg of plant tissues. Invert to mix the reagent well before dispensing.

1. Homogenize the tissue

Homogenize the animal tissue (~25 mg) or plant tissue (~50 mg) in 0.5 ml AquaRNA with a pestle-and-tube homogenizer (or a multi-channel bead beater). Move the pestle up and down while vortexing at top speed to fully homogenize the tissue. Add 2-3 drops (20-40 µl) of isopropanol to the homogenate to reduce foaming (*For RNA extraction from nuclease-rich specimens, such as liver and spleen tissues, add 1/3 vol (~167 µl) ProSink (#9030, order separately) to the homogenate, homogenize and vortex to mix well. Warning: Proteins can no longer be recovered after the homogenate has been mixed with ProSink.*), vortex and pour the homogenate into a 1.5-ml microfuge tube. Centrifuge at 14,000 xg for 5 min to pellet the tissue debris.

2. Recover the DNA/RNA

Transfer the clear lysate (~400 µl) to a new 1.5-ml microfuge tube. Add 0.8 vol (~320 µl) of isopropanol and vortex to mix. Centrifuge at 14,000 xg for 5 min to pellet the DNA/RNA. Decant to discard the supernatant (*Note: Proteins remain in the isopropanol supernatant and can be recovered by precipitation in 4 vol of acetone as described in Step 3. Recover the proteins*.). Gently fill the tube with 70% ethanol from a squirt bottle and then decant to discard the ethanol solution. Be sure to rinse the entire interior of the tube, including the inside of the cap. Repeat the ethanol rinse once. Tap the tube on a paper towel to remove residual ethanol, place the tube upside down on the paper towel to air dry the DNA/RNA pellet for 5-10 min. Add 400 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, and transfer the DNA/RNA solution to a new tube and store at –20 °C.

3. Recover the Proteins

Transfer 300 µl protein-containing isopropanol supernatant to a 2-ml microfuge tube. Add 4 vol (1.2 ml) acetone, vortex and centrifuge at 14,000 xg for 5 min to pellet the proteins. Decant to discard the supernatant, tap the tube on a paper towel to remove residual acetone. Immediately add 400 µl of ProMelt (#1115, order separately) 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 14,000 xg for 5 min to pellet any insoluble material. Transfer the protein solution to a new microfuge tube and use it directly in SDS-PAGE or store it at 4 or –20 °C (*Some SDS may precipitate out at these temperatures, it may be re-solubilized by incubating at 65 °C for 5-10 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 22 °C for 12 months. If AquaRNA becomes precipitated when exposed to low temperatures, you may incubate it at 37 °C for 15-20 min to resolubilize.

2. Do I need to use ProMelt and ProSink?

ProMelt (Item # 1115) is not needed, if you don't recover proteins. ProSink (Item # 9030) is not required for DNA/RNA extraction from bacteria, cultured cells, and most plant and animal tissues.

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

The 28S and 18S rRNA bands may migrate with the genomic DNA in a native 0.8% agarose gel. To see the 5S, 18S, and 28S rRNA bands. You can do a DNase I digestion to remove the genomic DNA before running the gel.

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

To troubleshoot RNase contamination, you may set up a DNase I digestion in 1x DNase buffer. Before adding DNase I, 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, your RNA sample may be contaminated. A good habit to prevent RNase contamination is to ensure that 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 RNA solution.

5. How should I remove the genomic DNA from my DNA/RNA preparation?

You may add 0.2 units of DNase I to 10-20 µl of DNA/RNA solution in 1x DNase buffer, 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.

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

Complete DNA removal may be difficult to achieve and unnecessary if you use intron-spanning primers for the PCR amplification. You may also 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], especially when intron-spanning is unavailable. In any case, you should always include a no-RT control in your amplification to confirm that your primers do not amplify the contaminating genomic DNA.