

AquaRNA RNA Purification Kit

**Order #
5001MT/5030MT**



Mo Bi Tec
MOLECULAR BIOTECHNOLOGY



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For research use only!!!



1. 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 are recovered from the cell lysate 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.

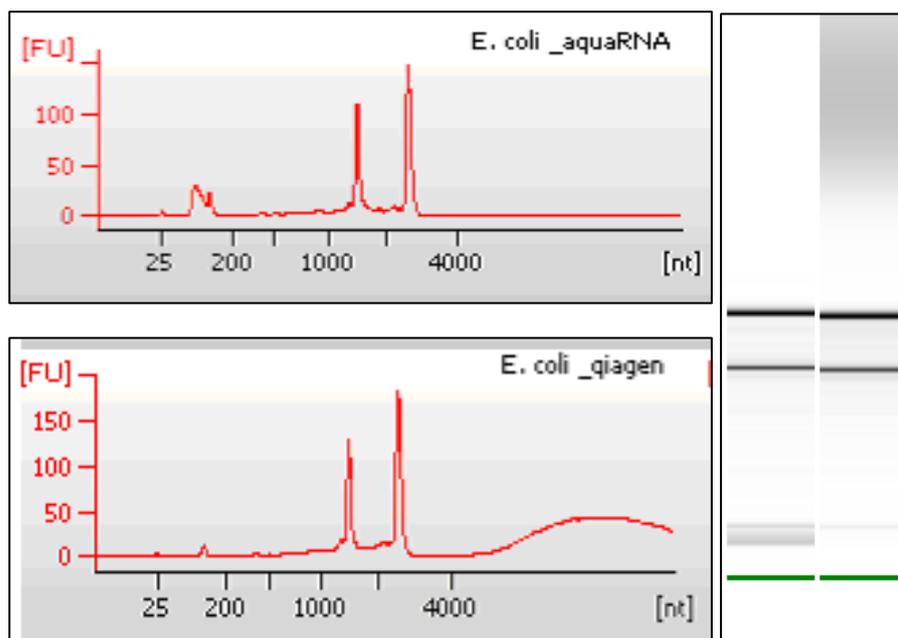


Figure 1. Total RNA was extracted from an *E. coli* culture, and the RNA integrity was measured with Agilent 2100 Bioanalyzer.

Kit Contents

5001MT	1 ml AquaRNA Solution	Instruction Manual
5030MT	30 ml AquaRNA Solution	Instruction Manual

Storage

Store tightly capped at 4 °C. Vortex to mix well before dispensing.

Note:

In addition to AquaRNA, we suggest ordering order ProSink (# 9030MT) for protein removal when extracting RNA from blood, liver, and spleen; and ProMelt (# 1115MT) for protein solubilization when extracting proteins.

2. Terms & Conditions

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

Product Warning: Contains guanidine thiocyanate. Harmful if swallowed. Causes irritation to skin, eyes, and respiratory tract. Do not mix with bleach.



3. AquaRNA Cell Protocol

This protocol uses 100 ml 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.

3.1 Harvest the cells

For eukaryotic cells: Centrifuge at 3,000 x g for 5 min to pellet 0.5-2 million cultured cells in a 1.5 ml reaction 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 x g for 1 min to pellet the cells. Aspirate to remove the medium and suspend the cells in 100 ml 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.

For animal tissues: Homogenize ~25 mg fresh or frozen or pulverized animal tissue in 500 µl of AquaRNA with a pestle-and-tube homogenizer (or ideally, homogenize the tissue with a mix of sixty 1.4 mm and six 2.8 mm ceramic beads, if a multi-channel bead beater is available), 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 1 - 2 drops (20 - 40 µl) of isopropanol to the sample to reduce foaming, and then vortex and pour the homogenate into a new microfuge tube. Centrifuge at 14,000 x g for 5 min to pellet the debris and recover the cleared lysate for protein and DNA/RNA extraction.

3.2 Extract the DNA/RNA

Add 100 ml 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-positive bacteria, use a bead beater to assist cell lysis). Optional: Centrifuge at 14,000 x g 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.*).

3.3 Pellet the DNA and RNA

Transfer the lysate (~90 µl) to a new 0.5-ml reaction tube. Add 0.8 vol (~72 µl) of isopropanol and vortex to mix. Centrifuge at 14,000 x g 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 x g for 5 min to pellet any insoluble material, and transfer the DNA/RNA solution to a new tube.



4. 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.

4.1 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 μ l) of isopropanol to the homogenate to reduce foaming, vortex and pour the homogenate into a 1.5-ml microfuge tube. Centrifuge at 14,000 x g for 5 min to pellet the tissue debris.

For RNA extraction from nuclease-rich specimens, such as liver and spleen tissues, add 1/3 vol (~167 μ l) ProSink (#9030, to be ordered 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.

4.2 Recover the DNA/RNA

Transfer the clear lysate (~400 μ l) to a new 1.5-ml reaction tube. Add 0.8 vol (~320 μ l) of isopropanol and vortex to mix. Centrifuge at 14,000 x g 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 μ l 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 x g for 5 min to pellet any insoluble material, and transfer the DNA/RNA solution to a new tube and store at -20 °C.

4.3 Recover the proteins

Transfer 300 μ l 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 μ l 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.).



5. 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. Invert to mix the reagent well before dispensing.

2. Do I need to use ProMelt and ProSink?

ProMelt (Item # 1115MT) is not needed, if you don't recover proteins. ProSink (Item # 9030MT) 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, for instance, 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.



6. Order Information, Shipping, and Storage

Order#	Product	Amount
5001MT	AquaRNA RNA purification Kit, 10 mini preps	1 ml
5030MT	AquaRNA RNA purification Kit, 300 mini preps	30 ml
shipped at RT; store at RT		

7. Related Products

Order#	Product	Amount
1115MT	ProMelt	15 ml
9030MT	ProSink	30 ml
1015MT	AquaPlasmid Solution, 75 minipreps	Kit
2010MT	AquaGenomic Solution, 30 preps	Kit
2030MT	AquaGenomic Solution, 300 preps	Kit

8. Contact and Support

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