AquaRNA™ Handbook

Mo Bi Tec
MOLECULAR BIOTECHNOLOGY

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Content

Introduction

AquaRNA™ is a multifunctional aqueous solution for DNA, RNA, and protein extraction. It functions to lyse the cells, to inactivate RNases, and to extract DNA, RNA, and proteins. DNA and RNA are readily recovered from the lysate by isopropanol precipitation, while proteins remain in the supernatant and can be recovered by acetone precipitation. It allows you to simultaneously isolate DNA, RNA, and proteins for analyses from the same cells, tissue, or organism, making it possible to get more out of limited and precious samples, or to save time, money and labor by doing three extractions at the same time.

Features

Innovative

One-of-a-kind aqueous solution that irreversibly inactivates RNases. If you have experienced the degradation of purified RNA during its storage or analysis and you know that SDS, guanidine thiocyanate, phenol/chloroform, and even autoclaving could not irreversibly inactivate RNases, you will appreciate this innovation.

Not only for RNA

AquaRNA™ can simultaneously extract DNA, RNA, and proteins from the same biological samples. If you are comparing two genomic profiles, such as normal vs. tumor tissues, why not compare their RNA expression profiles, and further, their protein expression profiles as well? In addition to maximizing the value of your invaluable samples, you might uncover new correlations and new differences.

Simple

Due to the multifunctionality of the AquaRNA™ solution, its protocol is very simple. After cell lysis, DNA/RNA are precipitated with isopropanol leaving proteins in the supernatant.

Easy

No columns, no resins, no slurries, no beads, no filters, no membranes, no needles, no cartridges, no manifolds, no HPLC, no ultracentrifuge.

No Nasty Organic Solvents

No phenol, no chloroform, no saturated butanol, no ethidium bromide, no cesium chloride, no 2-mercaptoethanol. Warning: Yes, it contains guanidine thiocyanate.

Pure

The purified RNA is no longer "very unstable" or tricky to work with, because it is no longer contaminated with endogenous RNases. Your RNA's integrity maintains during storage and subsequent analysis.

Scalable

One single AquaRNA™ Kit does all: mini, midi, maxi, and HTP; no need to purchase separate mini, midi, maxi, and HTP kits.

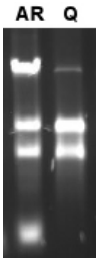
High Yield

Up to 100 µg of DNA, 50 µg of RNA, and 2000 µg of proteins can be isolated from 5 million mammalian cells or 50 mg animal tissue.

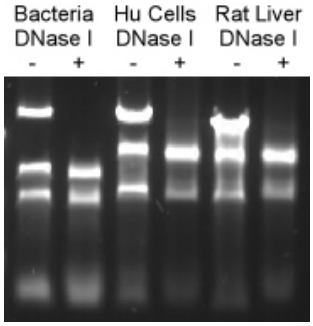
Economic

AquaRNA™ kit is priced at a fraction of other RNA isolation kits. Each miniprep uses 100 µl AquaRNA™ to extract DNA/RNA/proteins from 1 million cells.

Comparison

	Competitor Q	AquaRNA™
Isolation Mechanism	Selective Binding	Selective Extraction
Isolation Format	Solid Support	Aqueous Solution
Price (€/miniprep)*	3.89€	0.66€
Mercaptoethanol	Yes	No
Scalable	No	Yes
# of Solutions in the Kit	4	1
Hands-on Time (min)	30	30
Yield (µg RNA/mil cells)	10-20	10-20
RNase Contamination	?	No
Recover Small RNAs 	No	Yes
Co-Purify DNA/Proteins	Trace	Yes

*Comparison of list prices as of 05/2008



Comparison of total RNA isolated from different sources by AquaRNA™. Bacterial culture (0.5 ml), human cultured cells (500,000 cells), and rat liver tissue (20 mg) were processed using the AquaRNA™ Kit. Final DNA/RNA pellet was suspended in 100 µl DEPC water. Five microliter of each prep treated with or without DNase I was run in a non-denaturing 0.5% native agarose gel, showing the DNA, 28S (23S), 18S (16S), and 5S RNA bands.

Protocols

Total DNA/RNA/Protein Extraction Protocol

This protocol may be used to extract up to 100 µg of DNA, 50 µg of RNA, and 2000 µg of proteins from 5 million mammalian cells or 50 mg animal tissue, using 500 µl AquaRNA™. The DNA, RNA, and protein recovery is about 90% from the cleared lysate. You may scale up or down proportionately using different amount of starting materials. The extraction steps should be carried out on ice or at 4 °C.

1. Extract DNA/RNA/Protein

For eukaryotic cells:

1. Pellet ~5 million cultured cells by centrifugation at 12,000-20,000xg for 30 seconds.
2. Aspirate to discard the medium, leave behind ~50 µl of medium and vortex to resuspend the cells.
3. Transfer the cell suspension 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 to ensure no RNase escape inactivation.
4. Incubate on ice for 15 minutes, vortex vigorously for a minute at the end of the incubation.

For microbial cells:

1. Centrifuge 2.5 ml log-phase bacterial culture at 12,000-20,000xg for 30 seconds to pellet the cells.
2. Aspirate to discard the medium and suspend the cells in 500 µl of 1 mg/ml lysozyme (not supplied, use lyticase or equivalent for yeast cells) in TE buffer (pH 8.0, lysozyme will not be as effective at pH <8) and incubate on ice for >15 minutes, and vortex occasionally.
3. Transfer the cell suspension 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 to ensure no RNase escape inactivation.
4. Incubate on ice for 15 minutes, vortex vigorously for a minute at the end of the incubation.

For animal tissues:

1. Homogenize 50 mg fresh or frozen or pulverized animal tissue in 500 µl of AquaRNA™.
2. Transfer the homogenate to a 1.5 ml microfuge tube and centrifuge at 12,000-20,000xg for 2 minutes to pellet the debris.
3. Transfer the lysate (~450 µl) to a new tube and incubate on ice for 15 minutes, vortex vigorously for a minute at the end of the incubation.

For plant tissues:

1. Dissolve 1 g of hexadecyltrimethylammonium bromide (CTAB, not provided) in 10 ml of 10% isopropanol to obtain a 10% CTAB solution.
2. Add 1 volume of 10% CTAB to 9 volumes of AquaRNA™ and vortex vigorously to obtain an AquaRNA™ solution containing 1% of CTAB.
3. Homogenize 50 mg fresh or frozen or pulverized plant tissue in 500 µl of CTAB-AquaRNA™.
4. Transfer the homogenate to a 1.5 ml microfuge tube and centrifuge at 12,000-20,000xg for 5 minutes to pellet the debris.
5. Transfer the clear lysate (~400 µl) to a new tube with a pipette by tilting the tube to allow the clear lysate flow out underneath the creamy layer and incubate on ice for 15 minutes, vortex vigorously for a minute at the end of the incubation.

2. Precipitate DNA/RNA

1. Add 1 volume of 100% isopropanol to the lysate, vortex to mix well, centrifuge at 12,000-20,000xg for 5 minutes to pellet the DNA/RNA.
2. Transfer the supernatant to a new tube if protein recovery is desired and proceed with Step 3 (Precipitate Proteins) below. Otherwise flip the tube to discard the protein-containing supernatant.
3. Fill the tube with 75% ethanol by shooting the ethanol solution from a squirt bottle at the cap or sidewall of the tube, and then flip to discard the ethanol. Repeat the 75% ethanol rinse 2-3 times.
4. Flip to discard residual ethanol as completely as possible. Air-dry the pellet for a few minutes.
5. Add 100 µl of RNase-free water to the pellet and incubate at room temperature for 60 minutes to rehydrate the DNA/RNA.
6. Pipette up-and-down to break up the pellet and centrifuge at 12,000-20,000xg for 5 minutes to pellet any insoluble. Transfer the DNA/RNA solution to a new tube and store at –20 or -80 °C.

The purified DNA may be used for restriction digest, cloning, PCR, Southern blotting, chromosomal microarray, and ChIP-on-chip, etc. RNA contamination generally does not interfere with DNA analyses.

The purified RNA may be used for cDNA synthesis, RT-PCR, microarray, and Northern blotting, etc. with or without DNA removal. If DNA removal is desired, add DNase I buffer and RNase-free DNase I (not supplied) to the RNA solution, incubate at 37 °C for 15 minutes, precipitate the RNA with 1 volume of isopropanol, and heat-inactivate residual DNase I at 70 °C for 10 min. However, DNA removal is 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.

References:

Knuchel and Ansari (1997). Tailed RT-PCR for the quantitation of chloramphenicol acetyl transferase (CAT) mRNA, In "Methods in Molecular Medicine, Vol.XX - Quantitative PCR Protocols," Humana Press, (Chapter 18):1-11;
Hurteau and Spivack (2002). mRNA-specific reverse transcription-polymerase chain reaction from human tissue extracts. Anal Biochem. Aug 15;307(2):304-15; PMID 12202248;
Chen, *et al.* (2005) Real-time quantification of microRNAs by stem–loop RT–PCR. Nucleic Acids Research 33(20):e179 PMID 16314309

3. Precipitate Proteins

1. Transfer the protein-containing supernatant to a new tube following the isopropanol precipitation of DNA/RNA.
2. Add 4 volumes of acetone to the protein-containing supernatant and vortex to mix well.
3. Centrifuge at 12,000x for 5 minutes or 2,000xg for 15 minutes to pellet the proteins.
4. Flip the tube to discard the acetone supernatant into a waste container in a chemical hood.
5. Add 200 µl of 1% SDS solution to the pellet, pipette up-and-down and vortex to break up and fully suspend the pellet; transfer the solution to a 1.5 ml tube.
6. Centrifuge at 12,000-20,000xg for 5 minutes to pellet the insoluble, and transfer the protein solution to a new tube and store at 4 or -80 °C.

The purified proteins are ready for 1D-PAGE, 2D-PAGE, and Western blotting. They may also be used in protein microarray and other functional analyses after SDS is removed from the proteins by filtration or dialysis.

Blood Protocol

This protocol may be used to extract up to 60 µg DNA and 3 µg RNA from 500 ul of whole blood (fresh or frozen), using 500 µl of AquaRNA™. It requires a RBC Lysis Solution (Product # 4015MT, not included) to lyse the red blood cells prior to DNA/RNA extraction. Scale the volume of AquaRNA™ up or down proportionately for other starting volume of whole blood.

1. Harvest the Cells
Add 250 µl of RBC Lysis Solution to 500 µl whole blood in a 2 ml microfuge tube. Mix the contents by touch-vortex (a few seconds on and a split second off) for 20-30 times at top speed to lyse the red blood cells. Add 1 ml deionized water and vortex to wash the cells. Centrifuge at 12,000-20,000xg for 5 minute to pellet the white blood cells and nuclei. Aspirate to remove the supernatant. Add 1.5 ml deionized water to the cell pellet, vortex to fully disperse the pellet, and centrifuge to re-pellet the cells. Aspirate to remove the supernatant.

2. Extract the DNA/RNA
Add 500 µl of AquaRNA™ Solution to the cell pellet. Vortex vigorously to lyse the cells and extract DNA/RNA. Centrifuge at 12,000-20,000xg for 5 minute to pellet any insoluble. Transfer the clear lysate to a new 1.5 ml microfuge tube. Incubate on ice for 5-15 minutes.

3. Pellet the DNA/RNA
Add 1 volume (~500 µl) of 100% isopropanol to the lysate and mix the contents by vortexing for 30 seconds. Centrifuge at 12,000-20,000xg for 5 minutes to pellet the DNA/RNA. Decant to discard the supernatant. Fill the microfuge tube with 75% ethanol by shooting the ethanol solution from a squeeze bottle at the cap of the tube, and then decant to discard the ethanol. Repeat the 70% ethanol rinse 2-3 times. Flip the tube and tap it several times on a paper towel to remove residual ethanol. Add 100 µl of deionized water, incubate at room temperature 60 min to fully rehydrate the pellet, pipette up and down or vortex vigorously to suspend the DNA/RNA. Centrifuge at 12,000-20,000xg for 5 minutes to pellet the insoluble and transfer the DNA/RNA solution to a new tube. Store the DNA/RNA solution at –20 or –80 °C.

Dried Blood Protocol

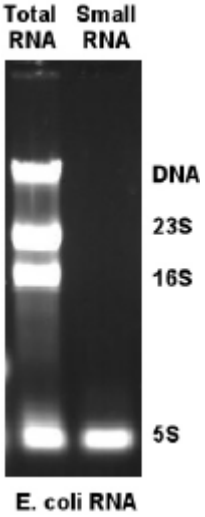
This protocol may be used to extract 200-400 ng of DNA from a dried bloodspot (from ~10-20 µl wet blood) or other biological samples such as bone marrow, using 100 µl of AquaRNA™.

1. Harvest the Cells
Add 100 µl of normal saline (0.9% NaCl) to the dried bloodspot on glass slide. Use the pipette tip to dislodge and transfer the sample to a 0.5 ml microfuge tube. (If the bloodstain is on other backing, scrap or cut it directly into a microfuge tube and add saline to extract the blood). Pipette and vortex to fully suspend the blood until no clump is visible. Centrifuge at 12,000-20,000xg for 2 minute to pellet the cells. Aspirate to remove the brown hemoglobin supernatant and leave behind the visible cell pellet.

2. Extract the DNA
Add 100 µl of AquaRNA™ Solution to the cell pellet. Vortex vigorously to lyse the cells and extract the DNA. Incubate at room temperature for 5 minutes and vortex again to ensure a homogeneous lysate with no visible insoluble.

3. Pellet the DNA
Add 1 volume (~100 µl) of 100% isopropanol to the lysate and mix the contents by vortexing for 30 seconds. Centrifuge at 12,000-20,000xg for 5 minutes to pellet the DNA. Decant to discard the supernatant. Fill the microfuge tube with 75% ethanol by shooting the ethanol solution from a squeeze bottle at the cap of the tube, and then decant to discard the ethanol. Repeat the 70% ethanol rinse 2-3 times. Flip the tube and tap it several times on a paper towel to remove residual ethanol. Add 20 µl of deionized water, incubate at room temperature for 15 min to fully rehydrate the pellet, pipette or vortex vigorously to suspend the DNA. Store the DNA solution at –20 or –80 °C.

Small RNA Protocol



This protocol can be used to isolate small RNAs, including 5S RNA, tRNA, microRNA, siRNA, or degraded RNA from various starting materials. It is essentially the same protocol as the other AquaRNA™ Protocols, except the addition of a 0.6 volume isopropanol (i.e., 40% final concentration) debris precipitation step to remove all other cellular components.

1. Extract the RNA

For bacteria:

Vortex to mix 500 µl of AquaRNA™ with 500 µl of lysozyme-treated bacteria (from 2.5 ml culture) in a 1.5 ml microfuge tube. Invert the tube a few times to wet the entire interior of the tube and incubate on ice for 30 min.

For cell cultures:

Vortex to mix 500 µl of AquaRNA™ with 50 µl cell suspension (~5 million cells) in a 1.5 ml microfuge tube. Invert the tube a few times to wet the entire interior of the tube and incubate on ice for 30 min.

For tissues:

Homogenize 50 mg of tissue in 500 µl of AquaRNA™ on ice and incubate on ice for 30 min. Vortex occasionally. Transfer the homogenate to a 1.5 ml microfuge tube.

2. Pellet the Debris

Add 0.6 volume (Do not add more than 0.7 volume or you will lose some small RNA.) of 100% isopropanol to the above lysate (for example, add 300 µl isopropanol to 500 µl lysate). Vortex and invert the tube to mix well. Centrifuge at 12,000-20,000xg for 10 min to pellet DNA and large RNA. Transfer the small RNA containing supernatant to a new microfuge tube.

3. Pellet the Small RNA

Add 0.4 volume (with respect to the volume of the recovered small RNA containing supernatant from step 2 above) of 100% isopropanol to the supernatant (for example, add 300 µl isopropanol to 750 µl recovered supernatant). Vortex to mix well. Centrifuge at 12,000-20,000xg for 10 minutes to pellet the small RNA. Flip to discard the supernatant. Fill the microfuge tube with 75% ethanol by shooting the ethanol solution from a squeeze bottle at the cap or sidewall of the tube, and then flip to discard the ethanol. Repeat the 75% ethanol rinse 2-3 times. Flip to discard residual ethanol as completely as possible. Air-dry the pellet for ~2-3 minutes. Add 100 µl of RNase-free water and vortex to solubilize the RNA. Store the RNA solution at -20 to -80 °C.

Order Information

AquaRNA™

Order #	Product	Amount
5001MT	AquaRNA™ RNA Purification Kit Small	1 mL (e.g. 10 MINI Preps)
5030MT	AquaRNA™ RNA Purification Kit Large	30 mL (e.g. 300 MINI Preps)

Store at RT. Only for research use!

Related Products

Order #	Product	Amount
1001MT	AquaPlasmid™ Plasmid DNA Purification Kit Trial	1 kit (e.g. 10 MINI Preps)
1010MT	AquaPlasmid™ Plasmid DNA Purification Kit Small	1 kit (e.g. 50 MINI Preps)
1030MT	AquaPlasmid™ Plasmid DNA Purification Kit Large	1 kit (e.g. 500 MINI Preps)

Order #	Product	Amount
2001MT	AquaGenomic™ Genomic DNA Purification Kit Trial	1 kit (e.g. 10 MINI Preps)
2010MT	AquaGenomic™ Genomic DNA Purification Kit Small	1 kit (e.g. 50 MINI Preps)
2030MT	AquaGenomic™ Genomic DNA Purification Kit Large	1 kit (e.g. 500 MINI Preps)

AquaRNA™ FAQ

1. Should I keep the AquaRNA™ kit in the freezer?
No, AquaRNA™ Solution is stable at 4 ° or 22 °C.

2. How does AquaRNA™ work?
AquaRNA™ is a multifunctional aqueous solution. It lyses the cells, inactivates the RNases, and extracts DNA, RNA, and proteins in one step. Total DNA/RNA are precipitated from the clear lysate with isopropanol and proteins in the supernatant are subsequently precipitated with acetone.

3. Do I need to add RNase Inhibitor to the final RNA solution?
No. AquaRNA™ is very effective in inactivating and removing RNases. You will discover that your RNA is no longer problematic and it is no longer tricky to work with RNA. You could even leave your RNA samples at room temperatures for days without noticing any RNA degradation.

4. How should I remove DNA from the RNA preparation?
If DNA removal is desired, you may use RNase-free DNase I to degrade the genomic DNA in the RNA preparation. However, DNA removal is unnecessary with the use of a simple and elegant 5' tailed RT-PCR.

[Knuchel and Ansari. Tailed RT-PCR for the quantitation of chloramphenicol acetyl transferase (CAT) mRNA, In "Methods in Molecular Medicine, Vol.XX - Quantitative PCR Protocols," Humana Press, (Chapter 18):1-11, 1997;
Hurteau and Spivack. mRNA-specific reverse transcription-polymerase chain reaction from human tissue extracts. Anal Biochem. 2002 Aug 15;307(2):304-15; PMID 12202248;
Chen, *et al.* Real-time quantification of microRNAs by stem–loop RT–PCR. Nucleic Acids Research 2005 33(20):e179 PMID 16314309]

5. Can I use AquaRNA™ with my Trizol® (Invitrogen) to inactivate RNases?
Yes, you could use AquaRNA™ in conjunction with other RNA isolation methods during extraction (1 vol. of AquaRNA™ and 1 vol. of phenol or Trizol or other lysis solution) to irreversibly inactivate RNases and you should at least double the RNA yield.

6. What QC tests do you use to certify your products?
Each lot is tested to ensure its performance and reliability in isolating DNA/RNA. An RNase assay is performed (incubating RNA prep in 1x DNase I buffer at 37 °C for 15 minutes and observing RNA integrity in 0.7% native agarose gel by electrophoresis) to ensure no RNase contamination.