AquaRNA Instruction Manual

General Information

Description

AquaRNA™ is a multifunctional aqueous reagent for DNA/RNA and protein extraction. This single solution will lyse the cells, inactivate degradative enzymes, and extract DNA/RNA and proteins. DNA/ RNA are recovered from the cell lysate by isopropanol precipitation, while proteins remain soluble in the AquaRNA-isopropanol solution and can be recovered by acetone precipitation or dialysis. 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™ Solution
Product #	5001, 5030
Size	5001: 1 ml AquaRNA for 10 minipreps from cultured cells 5030: 30 ml AquaRNA for 300 minipreps from cultured cells
MSDS	Available at www. multitargetpharm.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 liver, pancreas and spleen; and ProMelt (# 1115) for protein recovery.

Terms & Condition

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

Warranties and Liabilities: MultiTarget Pharmaceuticals 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. MultiTarget Pharmaceuticals MAKES NO WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING, WITHOUT LIMITATION, WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE.

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

Patents, Trademarks & Copyrights

AquaRNA™ is a trademark of MultiTarget Pharmaceuticals, LLC.

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.

1. Harvest the cells

(A) 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.
(B) For microbial cells: Centrifuge 0.25 ml log-phase culture at 12,000 xg for 1 min to pellet the cells and aspirate to remove the medium. Suspend the cells in 100 µl of 1 mg/ml lysozyme (not included, use lyticase for yeast cells) in TE buffer (*pH 8-9*) and incubate on ice for >15 minutes to breakdown the cell walls. Centrifuge to pellet the cells and aspirate to remove the supernatant.

2. Extract the DNA/RNA

Add 100 µl of AquaRNA to the cell pellet. Vortex to mix well. Incubate at room temperature for 5 min. Vortex and centrifuge at 12,000 xg for 5 min to pellet the cell debris. Transfer the clear lysate (80 µl) to a new 0.5-ml microfuge tube.

3. Pellet the DNA/RNA

(A) To pellet total DNA/RNA: Add 0.9 vol (72 µl) of isopropanol to the lysate. Vortex and centrifuge at 12,000 xg for 5 min to pellet the DNA/RNA. Transfer the protein-containing supernatant (100 µl) to a new tube for protein recovery.
(B) To pellet RNA and DNA differentially: Add 0.25 vol (20 µl) of isopropanol to the lysate. Vortex and centrifuge at 12,000 xg for 5 min to pellet large RNA. Transfer the supernatant to a new tube. Add an additional 50 µl of isopropanol, vortex and centrifuge for 5 min to pellet the DNA and small RNA. Transfer the protein-containing supernatant (100 µl) to a new tube for protein recovery.

4. **Rinse the DNA/ RNA pellet**

Gently fill up the tube and its lid with 70% ethanol from a squirt bottle and then decant to discard the ethanol solution. Repeat the ethanol rinse once. Tap the tube on a paper towel to remove residual ethanol and let the DNA/RNA pellet air-dry for 5-10 min. Add 100 µl of nuclease-free water to the pellet, vortex and/or pipette to solubilize the DNA/RNA pellet and centrifuge at 12,000 xg for 5 min to pellet any insoluble. Transfer the DNA/RNA solution to a new tube and store it at -20 °C.

5. Recover the proteins

Add 4 vol (400 µl) of acetone to the isopropanol supernatant (100 µl) obtained after DNA/RNA precipitation. Vortex and centrifuge at 12,000 xg for 5 min to pellet the proteins. Decant to discard the supernatant. Immediately add 100 µl ProMelt (#1150, order separately) to the wet protein pellet. Pipet and vortex to solubilize the proteins. Centrifuge to pellet any insoluble and save the protein solution for SDS-PAGE.

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.

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). Add 2-3 drops (20-30 µl) of isopropanol to the homogenate to reduce foaming and pour the homogenate into a 1.5-ml microfuge tube. Centrifuge at 12,000 xg for 5 min to pellet the tissue debris (*for plant samples, add 0.25 vol 1:1 isopropanol-diluted AquaRemove (#1208, order separately, e.g., add 100 µl isopropanol-diluted AquaRemove to 300 µl of homogenate), vortex and incubate at -20 °C for 10 min, centrifuge again to pellet the insoluble*).

2. Recover the DNA/RNA

Transfer the clear lysate (~300 µl) to a new 1.5-ml microfuge tube. Add 0.7 vol (~210 µl) of isopropanol. Vortex and centrifuge at 12,000 xg for 5 min to pellet the DNA/RNA. Decant to discard the supernatant (*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" below*). Gently fill up the tube and its lid with 70% ethanol from a squirt bottle and then decant to discard the ethanol solution. Repeat the ethanol rinse once. Tap the tube on a paper towel to remove residual ethanol and let the DNA/RNA pellet air-dry for 5-10 min. Add 400 µl of nuclease-free water to the pellet, vortex and/or pipette to solubilize the DNA/RNA. Vortex and centrifuge at 12,000 xg for 5 min to pellet any insoluble. Transfer the clear DNA/RNA solution to a new tube and store it 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) of acetone, vortex and centrifuge at 12,000 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 solubilize the proteins. Centrifuge at 12,000 xg for 5 min to pellet any insoluble. 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, centrifuge to pellet the insoluble or re-solubilized by incubating at 65 °C for 5-10 min prior to run the SDS-PAGE*).

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 the reagent.

2. 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 by RNase. A good habit to prevent RNase contamination is to ensure your gloves not touching the mouth of the tube when opening and closing the tube containing RNA solution.

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

You may add 0.2 U of DNase I to 10-20 µl of DNA/RNA solution in 0.5-1x DNase buffer, incubate at 22-37 °C for 20-30 min, and then run the sample in a 0.8% native agarose gel to confirm the completion of DNA digestion. To inactivate the DNase I, you may use Ambion's DNase removal reagent or inactivate the DNase I at 65 °C for 15 min. Additionally, large RNA, such as mRNA, may be differentially precipitated in 25% isopropanol from the AquaRNA lysate.

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 (may contain the insoluble pellet) 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% EtOH, and elute the DNA/RNA in 50 µl of deionized water or TE buffer.

5. 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.

MATERIAL SAFETY DATA SHEET (MSDS)

TRADE NAME: AquaRNA™ Solution
DATE OF ISSUE: March 1, 2015

SECTION I: PRODUCT AND MANUFACTURER INFORMATION

TRADE NAME: AquaRNA™ Solution

MultiTarget Pharmaceuticals, LLC
5050 Edison Ave Ste 214, Colorado Springs, CO 80915, USA
Telephone: 1-801-769-6586
DATE PREPARED: 03-01-2015

SECTION II: COMPOSITION / INFORMATION ON INGREDIENTS

SYNONYMS: AquaRNA™
CHEMICAL CHARACTERIZATION: Proprietary aqueous solution of Guanidine Thiocyanate and other chemicals.
HAZARDOUS COMPONENT: Guanidine Thiocyanate, CAS No.: 593-84-0.

SECTION III: HAZARD IDENTIFICATION

WARNING! HARMFUL IF SWALLOWED OR INHALED. CAUSES IRRITATION TO SKIN, EYES AND RESPIRATORY TRACT.
Health Rating: 2 - Moderate (Life)
Flammability Rating: 1 - Slight
Reactivity Rating: 1 - Slight
Contact Rating: 2 - Moderate
Lab Protective Equip: GOGGLES; LAB COAT; VENT HOOD; PROPER GLOVES
Storage Color Code: Green (General Storage).

SECTION IV: FIRST AID MEASURES

IF EYE CONTACT: Immediately flush eyes with copious amounts of water for at least 15 minutes. Assure adequate flushing of eyes by separating the eyelids with fingers.
IF SKIN CONTACT: Immediately wash skin with soap and copious amounts of water.
IF SWALLOWED: Wash out mouth with water provided person is conscious. Call a physician.

SECTION V: FIREFIGHTING MEASURES

EXTINGUISHING MEDIA: Use extinguishing media appropriate to surrounding fire conditions.
SPECIAL FIREFIGHTING PROCEDURES: Wear self-contained breathing apparatus and protective clothing to prevent contact with eyes and skin.

SECTION VI: ACCIDENTAL RELEASE MEASURES

PRECAUTIONARY MEASURES: Wear self-contained breathing apparatus, chemical safety goggles, rubber boots, and heavy rubber gloves.
CLEAN-UP PROCEDURES: Absorb on sand or vermiculite and place in closed container for disposal. Ventilate area and wash spill site after material pick-up is complete.

SECTION VII: HANDLING AND STORAGE

STORAGE: Store tightly closed at 4-25 °C.

SECTION VIII: EXPOSURE CONTROLS AND PERSONAL PROTECTION

Chemical safety goggles. Rubber gloves. Safety shower and eye bath. Wash thoroughly after handling. Do not get in eyes, on skin, or on clothing.

SECTION IX: PHYSICAL AND CHEMICAL PROPERTIES
PHYSICAL STATE / FORM: Liquid
COLOR: Light yellow
ODOR: None
pH: (20 °C) N/V
VISCOSITY: (20 °C) N/A
MELTING POINT: N/A
BOILING POINT: N/A
IGNITION TEMPERATURE: N/A
FLASHPOINT: N/A
EXPLOSION LEVEL: N/A
VAPOR PRESSURE: (20 °C) N/A
SPECIFIC GRAVITY: (20 °C) N/A
SOLUBILITY IN WATER: (20 °C) Soluble

SECTION X: STABILITY AND REACTIVITY
Stable under normal conditions of use, temperature and pressure.
SUBSTANCES TO BE AVOIDED: Strong oxidizing agents, strong acids, and acid chlorides
HAZARDOUS, COMBUSTION, OR DECOMPOSITION PRODUCTS: N/A

SECTION XI: TOXICOLOGICAL INFORMATION
TOXICITY DATA: N/A
INHALATION: May be harmful by inhalation.
EYE CONTACT: May cause eye irritation.
SKIN CONTACT: May cause skin irritation, may be harmful by skin absorption.
INGESTION: May be harmful if swallowed.
PROLONGED EXPOSURE: N/A
CHRONIC EFFECTS: N/A
RTECS NUMBER: For Guanidine Thiocyanate: XL1225000
ADDITIONAL INFORMATION: THE CHEMICAL, PHYSICAL, AND TOXICOLOGICAL PROPERTIES HAVE NOT BEEN THOROUGHLY INVESTIGATED. Additional harmful properties cannot be ruled out. The product should be handled with the normal caution accorded chemicals.

SECTION XII: ECOLOGICAL INFORMATION
N/A

SECTION XIII: DISPOSAL CONSIDERATIONS
There are no uniform regulations for the disposal of chemicals or residues. Dispose of container and unused contents in accordance with federal, state and local requirements.

SECTION XIV: TRANSPORT INFORMATION
DOT: None of the components are regulated.

SECTION XV: REGULATORY INFORMATION
CAS # 593-84-0: Not listed on SARA, TSCA, EPA, IARC, NTP, TLV, MAK, NIOSH-Ca, OSHA-Ca

SECTION XVI: OTHER INFORMATION
DATE OF PREPARATION: March 1, 2015.
DISCLAIMER: For research use only. The above information is believe to be correct but does not purport to be all-inclusive and should be used only as a guide. MultiTarget Pharmaceuticals shall not be held liable for any damages or other consequences resulting from handling or from contact with the above product.