AquaPreserve Instruction Manual

General Information

Description

AquaPreserve™ is a multifunctional aqueous reagent for DNA/RNA/protein preservation and extraction. It may be used to streamline biospecimen collection, stabilization, transport, storage, distribution, and DNA/RNA/protein extraction. By streamlining the entire biospecimen workflow, AquaPreserve can reduce pre-analytical variability, increase data reproducibility and reliability. AquaPreserve can stabilize and extract total DNA/RNA/proteins from whole blood, plasma, saliva and other liquid or solid biospecimens. AquaPreserve is the only reagent that can extract intact RNA from frozen whole blood samples collected in common anticoagulants.

Specification

Product Name	AquaPreserve™ Solution
Product #	8001, 8030, 8060
Size	8001: 1 ml AquaPreserve for 4 minipreps from whole blood sample 8030: 30 ml AquaPreserve for 120 minipreps from whole blood sample 8060: 60 ml AquaPreserve for 240 minipreps from whole blood sample
MSDS	Available at www. multitargetpharm.com
Storage	Store tightly capped at 22 °C. Vortex to mix well before dispensing.
Note	In addition to AquaPreserve, please order ProSink (# 9030) for blood DNA and RNA extraction; 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

AquaPreserve™ is a trademark of MultiTarget Pharmaceuticals, LLC.
©2018 Multitarget Pharmaceuticals, LLC. All rights reserved.

AquaPreserve Blood Biobanking Protocol

AquaPreserve combines blood DNA/RNA/protein preservation with extraction. It may be used to streamline blood collection, stabilization, transport, storage, distribution, and DNA/RNA/protein extraction, and reduce specimen pre-analytical variability.

1. Donors

The laws and regulations governing human subject protection and privacy must be strictly adhered to. Informed consent must be obtained from all donors. Patient selection should represent a broad range of human diseases but focus may be placed on diseases with unmet need, having suspected genetic etiology, and patients with well annotated EMRs. All donors must be de-identified.

2. Collection

Fresh anticoagulated blood sample may be mixed with one blood volume of AquaPreserve immediately following the blood draw to stabilize the blood sample. Alternatively it is possible to pre-inject half a volume of AquaPreserve into a vacutainer tube with a fine needle and subsequently use the vacutainer containing AquaPreserve to draw fresh blood directly. Finally leftover blood samples stored at 4 °C up to 7 days after their prescribed laboratory tests may also be collected and mixed with one volume of AquaPreserve for later analysis.

3. Transport

The AquaPreserve-stabilized blood samples may be barcoded, linked to their de-identified donors' EMRs, and transported to the biobank overnight in wet ice.

4. Storage

Upon arriving at the biobank, the AquaPreserve-stabilized blood samples may be stored at 22 °C overnight or 4 °C for 2 weeks or at -80 °C indefinitely before DNA/RNA/protein extraction. Unlike frozen plain blood, AquaPreserve-stabilized blood samples can tolerate accidental sample thawing, such as power outage or freezer breakdown.

5. Distribution

AquaPreserve-stabilized blood samples can be thawed and aliquoted for QC/QA testing or distribution to other investigators. In contrast, freeze-and-thawing is strictly prohibited for frozen plain blood as blood RNA will be degraded by freeze-and-thawing.

6. Extraction

The end-users may extract total blood DNA/RNA (require the purchase of ProSink #9030) or proteins (require the purchase of ProMelt #1115) from AquaPreserve-stabilized blood samples for downstream analyses.

AquaPreserve Blood DNA/RNA Extraction Protocol

This protocol uses 0.25 ml AquaPreserve (#8060) and 0.125 ml ProSink (#9030, ordered separately) to extract DNA (~12 µg) and RNA (~250 ng) from 0.25 ml fresh or frozen human blood collected in regular anticoagulants. This protocol may also be used to extract DNA/RNA from saliva and other liquid biospecimens.

1. **Lyse the blood cells**. Add 0.25 ml of AquaPreserve to 0.25 ml of fresh or frozen whole blood in a 1.5-ml microfuge tube. Vortex to thaw the blood (*Do not thaw frozen blood without mixing with AquaPreserve or the RNA will be degraded during blood thawing. However, for blood DNA extraction only, the blood sample should be thawed and incubated at 22 °C for 20 min to degrade the RNA prior to mixing with AquaPreserve.*). Incubate at 22 °C for 15 min. Shake the tube vigorously to break up the blood clot and centrifuge at 12,000 xg for 5 min to pellet the debris.

2. Pellet the proteins. Add 0.125 ml of ProSink to the crude lysate. Invert the tube and touch vortex a few times to mix well. Incubate at 22 °C for >30 min (*Blood DNA is now stable at 4-22 °C for months, and blood RNA is stable at 4 °C for 2 weeks and at 22 °C for 7 days).* Centrifuge at 12,000 xg for 5 min to pellet the proteins.

3. Pellet the DNA/RNA. Transfer the supernatant (~0.7 ml) to a new 1.5-ml microfuge tube. Add 0.9 vol (~0.63 ml) of isopropanol. Touch vortex a few times to mix well. Centrifuge at 12,000 xg for 5 min to pellet the DNA/RNA. Decant to discard the supernatant.

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.

5. Solubilize the DNA/RNA pellet. Add 100 µl of deionized water to the DNA/RNA pellet. Vortex and/or pipet to solubilize the DNA/RNA. Incubate at 22 °C for 10 min. Centrifuge again to pellet any insoluble and transfer the clear DNA/RNA solution to a new tube. Store at –20 °C.

Table 1. Use the volume ratio of 1:1:0.5 (blood:AquaPreserve:ProSink) for other extraction scales

	Micro	**Mini**	**Midi**	**Maxi**
Blood (µl)	50	250	2,000	4,000
AquaPreserve (µl)	50	250	2,000	4,000
ProSink (µl)	25	125	1,000	2,000
Centrifuge tubes	0.6-ml	1.5-ml	15-ml	15-ml
DNA yield (µg)	2-3	12-15	100-130	200-250
RNA yield (ng) *	50	250	2,000	4,000
Number of extractions	1,200	240	30	15

* RNA yield from human blood. For mouse blood, RNA yield may be 2500 ng/50 ul blood.

AquaPreserve Blood Protein Extraction Protocol

Total blood proteins may be extracted from fresh or frozen whole blood sample using the following protocol. You will need to order ProMelt (#1115) separately to solubilize the protein pellet obtained by acetone precipitation for SDS-PAGE analysis.

1. Lyse the blood cells

Add 0.1 ml of AquaPreserve to 0.1 ml of fresh or frozen whole blood in a 1.5-ml microfuge tube. Vortex to mix well.

2. Pellet the DNA/RNA

Add 0.9 vol (0.18 ml) of isopropanol, vortex for 60 sec, and centrifuge at 12,000 xg for 5 min to pellet the blood DNA/RNA while leave the proteins soluble in the supernatant.

3. Pellet the proteins

Transfer the protein-containing supernatant (0.3 ml) to a new 1.5-ml microfuge tube. Add 4 vol (1.2 ml) of acetone, vortex for 60 sec, and centrifuge at 12,000 xg for 5 min to pellet the proteins.

(Note: *Proteins may also be recovered by dialysis of the protein-containing supernatant, instead of using acetone precipitation*).

4. Solubilize the proteins

Decant to discard the supernatant, tap the tube on a clean paper towel to remove residual acetone. Immediately add 0.5 ml of ProMelt to the wet protein pellet, pipette and vortex to suspend the protein pellet. Incubate at 22 °C for 15 min to solubilize the proteins. Vortex and centrifuge at 12,000 xg for 5 min to pellet any insoluble. Transfer the protein solution to a new microfuge tube and store it at 4 or –20 °C.

(Note: *Some SDS may precipitate out at low temperature, which may be removed by centrifugation. Alternatively the precipitates may be re-solubilized by incubating the sample at 65 °C for 10 min*).

AquaPreserve Buffy Coat Protocol

If you need to recover the plasma for other assays or extract DNA/RNA/proteins from a large volume of blood, you may prepare buffy coat from fresh whole blood for DNA/RNA/protein extraction to reduce the consumption of the extraction reagents. The protocol below is for processing ~2 ml of whole blood to obtain ~200 µl of buffy coat. If you need to process larger volume of whole blood in the original vacutainer (5-10 ml), you may scale up the reagent volumes proportionally.

1. Prepare the buffy coat

Centrifuge 2 ml of anticoagulated whole blood at 300 xg for 10 min at room temperature. Remove some plasma (~0.6-0.7 ml) without disturbing the buffy coat. Set the pipette at 100-µl and carefully suck up the grayish buffy coat while slowly moving the tip across the interface and taking up as little RBC as possible. Transfer the buffy coat to a 1.5-ml microfuge tube. Repeat it by taking 100 µl of plasma just above the interface. The total volume of buffy coat recovered is about 1/10 of the blood volume, that is, ~200 µl.

2. Lyse the blood cells

Add one volume (~200 µl) of AquaPreserve to the buffy coat. Vortex to mix well.

3. Recover the DNA/RNA

Add 0.9 volumes (~360 µl) of isopropanol to the cell lysate. Vortex and centrifuge at 12,000 xg for 5 min to pellet the DNA/RNA. Transfer 0.4 ml protein-containing supernatant to a 2-ml tube for protein recovery. Decant to discard the remaining suspernatant from 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 deionized water to the DNA/RNA pellet, pipet and vortex to suspend the DNA/RNA. Incubate at room temperature for 10 min and centrifuge again to pellet any insoluble. Transfer the clear DNA/RNA solution to a new tube and store it at –20 °C.

4. Recover the proteins

Add 4 volumes (1.6 ml) of acetone to the isopropanol supernatant obtained after DNA/RNA precipitation (*Proteins may also be recovered by dialysis instead of acetone precipitation*). Vortex to mix well. Centrifuge at 12,000 xg for 5 min to pellet the proteins. Decant to discard the supernatant. Immediately add 100 µl of 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.

(*Note: If the buffy coat contains large amount of RBC, you may need to use 2 volumes of AquaPreserve for the extraction or try various volume of ProSink (#9030) for protein precipitation to reduce hemoglobin contamination of the recovered DNA/RNA*.)

AquaPreserve Plasma Protocol

This protocol can be used to prepare ~10-20 ng of cell-free circulating DNA and ~50 mg proteins from 0.9 ml of plasma (or serum), using 0.9 ml of AquaPreserve solution. You may scale up or down the AquaPreserve volume proportionally (1:1) as needed for other starting plasma volumes.

1. **Prepare the plasma**

Prepare the plasma (or serum) from fresh whole blood using standard methods. Transfer 0.9 ml plasma to a 2-ml microfuge tube.

2. Extract the plasma DNA

Add 1 volume (0.9 ml) of AquaPreserve solution to the plasma. Vortex to mix well.

3. Pellet the plasma DNA

Divide the AquaPreserve-plasma solution into two 2-ml microfuge tubes. Add 0.9 volume (0.81 ml) of isopropanol to each tube. Vortex to mix well. Centrifuge at 12,000xg for 5 minutes to pellet the DNA.

4. Recover the plasma proteins

Transfer the isopropanol supernatant (~1.6 ml) to a new tube for protein recovery by acetone precipitation (or dialysis). To pellet the proteins, add 4 vol (~6.4 ml) of acetone to the isopropanol supernatant. Vortex and centrifuge at 12,000xg for 5 min to pellet the proteins. Decant to discard the supernatant into a waste container in a chemical hood. Add 100 µl of ProMelt (#1150) to the protein pellet, pipet and vortex to solubilize the proteins. Centrifuge to pellet any insoluble and save the protein solution for SDS-PAGE.

5. Rinse the plasma DNA 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.

6. Solubilize the plasma DNA pellet

Add 25 µl of TE buffer or deionized water to the DNA pellet, pipette and vortex to solubilize the DNA. Centrifuge at 12,000xg for 5 min to pellet any insoluble. Transfer the DNA solution to a new tube and store at 4 or –20 °C.

AquaPreserve QIAcube Protocol

To use AquaPreserve with Qiagen's QIAcube for automated blood DNA/RNA purification, the following steps may be performed prior to loading the samples into QIAcube for automated DNA/RNA purification.

1. Set up the QIAcube

Close the QIAcube door, and switch on the QIAcube with the power switch. A beeper sounds and the startup screen appears. The instrument automatically performs initialization tests.Open the QIAcube door, and load the necessary reagents and plasticware into the QIAcube. To save time, loading can be performed during one or both of the following 10-minute centrifugation steps.

3. Remove blood proteins

Mix 2.5 ml whole blood sample with 2.5 ml AquaPreserve in a 10-15-ml centrifuge tube. Vortex to mix well and incubate at room temperature for 10 min to lyse the blood cells. Add 1.25 ml ProSink (#9030 order separately) to the tube and vortex to mix well. Incubate at RT for 10 min to precipitate the proteins. Centrifuge for 10 min at 8,000–12,000 x g using a swing-out rotor to pellet the proteins.

4. Pellet the blood DNA/RNA

Transfer the supernatant to a new 10-15-ml centrifuge tube. Add 0.9 vol of isopropanol (e.g., for 4 ml of supernatant, add 3.6 ml of isopropanol) and vortex to mix well. Incubate at room temperature for 10 min to precipitate the DNA/RNA. Centrifuge for 10 min at 8,000-12,000 xg to pellet the DNA/RNA.

5. Rinse the blood DNA/RNA pellet

Decant to discard the entire supernatant. 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.

6. Solubilize the blood DNA/RNA pellet

Add 350 µl Qiagen's Buffer BM1 and vortex until the pellet is visibly dissolved. And then continue with the QIAcube protocol to purify the blood DNA and blood RNA.

Frequently Asked Questions

Please read through these questions carefully. The answers provide additional tips and useful information for the successful use of AquaPreserve.

1. How should I store the AquaPreserve solution?

It may be stored at 22 °C for 12 months. If AquaPreserve becomes precipitated when exposed to low temperature, you may incubate it at 37 °C for 15-20 min to resolubilize the reagent.

2. Can we use AquaPreserve to preserve solid tissue samples?

Yes, you may preserve solid tissue samples in 2 volumes of AquaPreserve for biobanking. The tissue/organ may be immersed in AquaPreserve to keep its gross anatomy, or homogenized and aliquoted for storage and distribution to researchers for DNA/RNA/protein extraction. ProSink is not needed for DNA/RNA extraction from most solid tissues other than the RNase-rich liver, pancreas and spleen tissues.

3. How should I remove the genomic DNA from the 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 digested 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.

4. Why did my DNA/RNA solution show 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 and load it into the spin column, centrifuge to allow DNA/RNA binding to the silica membrane, wash the column with 0.5 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 or 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: AquaPreserve™ Solution
DATE OF ISSUE: March 1, 2015

SECTION I: PRODUCT AND MANUFACTURER INFORMATION

TRADE NAME: AquaPreserve™ 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: AquaPreserve™
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 °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.