AquaPreserve Instruction Manual

General Information

Description

AquaPreserve™ is a multifunctional 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 extracts total DNA/RNA/proteins from whole blood; it recovers both cellular and cell-free circulating DNA/RNA/proteins in the whole blood, therefore, maximizing the scientific value and utilities of the biospecimens.

Specification

Product Name	AquaPreserve™ Kit
Product #	8001, 8060
Size	8001: 1 ml; 8060: 60 ml (sufficient for 240 mini, 30 midi, 15 maxi preps)
Kit Contents	8001: 1 ml AquaPreserve Solution, User Manual 8060: 60 ml AquaPreserve Solution, User Manual
MSDS	Available at www.aquaplasmid.com
Storage	Store tightly capped at 22 °C. Vortex the reagent 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 extraction.

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

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.

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 and shake (*holding the tube in your hand while shaking it will help the AquaPreserve solution penetrating into the frozen blood*) to thaw the blood (*Do not thaw the 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, 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 lysate. Invert the tube and touch vortex a few times to mix the lysate and ProSink without dislodging the debris pellet. 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 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 *(If needed, centrifuge the clear lysate again to remove any carried-over debris).* Add 1 vol (~0.7 ml) of isopropanol. Touch vortex a few times to mix well. Centrifuge at 12,000 xg for 5 min to pellet the DNA/RNA.

4. Rinse the DNA/RNA pellet. Decant to discard the supernatant (*or save it for small molecules analysis*). Gently shoot 70% ethanol from a squirt bottle to fill up the tube (*Do not shoot the alcohol solution directly onto the pellet.*), and decant to discard the alcohol solution (*Make sure the DNA/RNA pellet remains in place before pouring off the alcohol solution.*). Repeat the isopropanol rinse once. Tap the tube on a paper towel to remove residual liquid and leave it upside down to air dry the DNA/RNA pellet 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

AquaPreserve for total blood DNA/RNA biobanking

AquaPreserve combines blood DNA/RNA preservation with extraction. It may be used to streamline blood collection, stabilizaton, transport, storage, distribution, and DNA/RNA extraction, and reduce specimen pre-analytical variability. Furthermore, AquaPreserve extracts both cellular and cell-free circulating DNA/RNA from either fresh or frozen whole blood samples, maximizing the value and utilities of the blood specimens.

1. Stabilize the blood sample. Fresh blood samples should be immediately stabilized with AquaPreserve upon arrival at the laboratory. Un-stabilized blood samples should be stored at –80 °C or at –20 °C. To stabilize the blood samples, transfer an aliquot of the blood sample (e.g., 0.25, 0.5, 2, or 4 ml) to a tube containing an equal volume of AquaPreserve. Vortex to mix well. Store the AquaPreserve stabilized blood samples at -80 °C until blood DNA/RNA/protein extraction. If the blood samples will be shipped at ambient temperature and are intended for RNA extraction, add 0.5 volume of ProSink (e.g., for 1 ml AquaPreserve, use 0.5 ml ProSink) to the AquaPreserve-lysed blood sample, shake or vortex to mix well.

2. Transport the blood sample. If the blood samples are for DNA extraction only, they can be shipped and stored at ambient temperature after being stabilized with AquaPreserve. If they will be used for RNA extraction, the blood samples should be stabilized with AquaPreserve and ProSink for shipping at ambient temperature or on cold gel packs.

3. Store the blood sample. AquaPreserve and ProSink stabilized blood samples may be stored at 22 °C for 1-5 days or at 4 °C for 1-2 weeks. For long-term storage, the stabilized blood samples should be stored at –80 °C. Do not store AquaPreserve/ProSink stabilized blood samples at -20 °C as it reduces RNA yield significantly.

4. Extract the DNA and RNA.

(1) To extract DNA/RNA from AquaPreserve-stabilized blood. At the time of DNA/RNA extraction, simply thaw the AquaPreserve stabilized blood sample, centrifuge to pellet the debris, and then add 0.25 volume of ProSink (e.g., for 1 ml AquaPreserve-lysed blood sample, add 0.25 ml ProSink), vortex to mix well, centrifuge to pellet the proteins (*save the pellet for protein recovery, if desired*) and recover the clear lysate for DNA/RNA precipitation with 1 volume of isopropanol.

(2) To extract DNA/RNA from frozen blood. If the blood sample is contained in a tube 3x of the sample volume, add one volume of AquaPreserve to the frozen blood, shake and vortex vigorously to thaw the blood in AquaPreserve. If the frozen blood is in original vacutainer, the bottom of the vacutainer may be cut off so that the frozen blood pellet can be pushed out into a 50-ml conical tube preloaded with one volume of AquaPreserve (*Note: If RNAis not needed, you may thaw the blood sample, vortex to mix well and take an aliquot for DNA extraction.*). Shake and vortex vigorously to thaw the frozen blood in AquaPreserve. Centrifuge to pellet the debris. And then add 0.25 volume of ProSink (e.g., for 1 ml AquaPreserve-lysed blood sample, add 0.25 ml ProSink), shake and vortex to mix well. Incubate at 22 °C for >30 min, and then centrifuge to pellet the proteins (*save the pellet for protein recovery, if desired*) and recover the clear lysate for DNA/RNA precipitation with 1 volume of isopropanol.

AquaPreserve Blood Protein Extraction Protocol

Total blood proteins may be extracted from fresh or frozen whole blood sample using one of the following two protocols. The first protocol "Protein precipitation with ProSink" recovers blood proteins from ProSink precipitated protein pellet left over from blood DNA/RNA extraction. The second protocol "Protein precipitation with acetone" recovers blood proteins from AquaPreserve-lysed blood directly by acetone precipitation, independent of blood DNA/RNA extraction. The first protocol is convenient and streamlines blood DNA/RNA/protein extraction. However, the solubility of the proteins is lowered after being treated with ProSink. If the concentration of your target protein is too low to be detected with the first protocol, you may use the second protocol for protein precipitation to ensure all the proteins can be solubilized. You may further increase the cellular protein concentration by extracting the proteins from the buffy coat.

A. Protein precipitation with ProSink

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. Shake and vortex to mix well and incubate at 22 °C for 15 min. Centrifuge at 12,000 xg for 5 min.

2. Pellet the proteins. Add 0.125 ml of ProSink to the centrifuged sample. Shake and vortex to mix well. Incubate at 22 °C for 30 min. Centrifuge at 12,000 xg for 5 min to pellet the proteins.

3. Solubilize the proteins. Remove the supernatant for DNA/RNA precipitation. Add 1 ml of ProMelt (#1115, order separately) to the wet protein pellet. Pipet up and down to suspend the protein pellet. Take 10 µl of the protein suspension and mix with 90 µl of ProMelt. Vortex and incubate at 37 °C for 10 min to solubilize the proteins completely for SDS-PAGE.

B. Protein precipitation with acetone

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 or shake to mix well.

2. Pellet the DNA/RNA. Add 0.7 vol (0.14 ml) of isopropanol, vortex for 60 sec, and centrifuge at 12,000 xg for 5 min to pellet the blood DNA/RNA.

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.

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 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 at 4 or –20 °C (*Some SDS may precipitate out at low temperatures, however, it will not interfere with SDS-PAGE. Alternatively it may be re-solubilized by incubating at 65 °C for 10 min.*).

AquaPreserve Buffy Coat DNA/RNA/Protein Extraction 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 will simply 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 0.8 volume (~320 µl) of isopropanol to the cell lysate. Vortex to mix well. Centrifuge at 12,000 xg for 5 min at room temperature to pellet the DNA/RNA. Transfer 0.4 ml protein-containing supernatant to a 2-ml tube for protein recovery. Remove the remaining supernatant from the DNA/RNA pellet as much as possible. Fill up the microfuge tube with 50% isopropanol and quickly decant to discard the isopropanol solution. Repeat the isopropanol rinse once. Tap the tube on a clear paper towel to remove residual isopropanol and leave the tube up side down to air dry the DNA/RNA pellet for 5-10 min. Add 100 µl of deionized water to the pellet and vortex to suspend the DNA/RNA pellet. Incubate at room temperature for 10 min and centrifuge again to pellet any insoluble. Transfer the DNA/RNA solution to a new tube and store at –20 °C.

4. Recover the proteins

Add 4 volumes (1.6 ml) of acetone to the isopropanol supernatant obtained after DNA/RNA precipitation. Shake or 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. The protein solution may be loaded directly to 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.*)

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 temperatures, you may incubate it at 37 °C for 15-20 min to resolubilize it.

2. When do I need to use ProMelt and ProSink?

ProMelt (Item # 1115) is not needed, if you will not recover the proteins. ProSink (Item # 9030) is a protein-precipitating reagent and it is required for blood DNA/RNA extraction.

3. How should I thaw 1 ml frozen blood in a 1.5-ml tube?

Ideally, the fresh blood sample is aliquoted in tubes 3x of the blood sample volume, or pre-mixed with AquaPreserve and ProSink prior to freezing. However, to process existing 1-ml frozen blood sample in a 1.5-ml tube, you may either cut open the tube to retrieve the frozen blood pellet or use 0.4 ml of AquaPreserve to partially thaw the frozen blood repeatedly and transfer it to a large tube.

4. Why didn't I see the 28S and 18S rRNA bands in the gel?

The 28S and 18S rRNA bands may migrate with the genomic DNA in a native 0.8% agarose gel. If you add some salts (e.g., 30 μM NaOAc, pH unadjusted) to the loading dye, you may get a better separation of the DNA and RNA bands. However, it would be better to do a DNase I digestion to remove the DNA before running the gel.

5. 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, 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. To inactivate the DNase I, you may use Ambion's DNase removal reagent or 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 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.