# 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 can extract total DNA/RNA/proteins from blood, saliva and other liquid biospecimens; it may recover both cellular and cell-free circulating DNA/RNA/proteins in a specimen, and thus maximizing the scientific value and utilities of the biospecimens. AquaPreserve is the only reagent that can extract intact RNA from frozen whole blood samples collected in common anticoagulants.

### 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<br>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. This protocol may also be used to extract DNA and 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 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. 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. 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.

**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 ethanol solution directly onto the pellet.*), and decant to discard the ethanol solution (*Make sure the DNA/RNA pellet remains in place before pouring off the ethanol solution.*). Repeat the ethanol rinse once (use centrifuge if the pellet is loosening). 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.

**2. Transport the blood sample**. If the blood samples are to be used for DNA extraction only, they may be shipped and stored at ambient temperature after being stabilized with AquaPreserve. For RNA extraction, AquaPreserve-stabilized blood samples should be shipped within 24 hours; however, if the transit time will be longer than 1-2 days at ambient temperature, you should mix AquaPreserve-lysed blood sample with 0.25 volume of ProSink (e.g., add 0.25 ml ProSink to 1 ml AquaPreserve-lysed blood sample, vortex or shake to mix well).

**3. Store the blood sample**. AquaPreserve/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., add 0.25 ml ProSink to 1 ml AquaPreserve-lysed blood sample), 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 0.9 volume of isopropanol.

**(2) To extract DNA/RNA from frozen blood**. If the blood sample is 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 (*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 0.9 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 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 0.9 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.

**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. (*Proteins may be recovered by dialysis instead of 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 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 temperature, 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 0.9 volume (~360 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 70% ethanol and quickly decant to discard the ethanol solution. Repeat the ethanol rinse once. Tap the tube on a clear paper towel to remove residual ethanol 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 (*Proteins may be recovered by dialysis instead of acetone 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.*)

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

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

3. Transfer 0.9 ml of the mixture to another 2-ml microfuge tube. Add 0.9 volume (0.81 ml) of isopropanol to each tube containing the 0.9 ml plasma/AquaPreserve mixture. Vortex to mix well. Centrifuge at 12,000xg for 5 minutes to pellet the DNA.

4. Transfer the isopropanol supernatant (~1.6 ml) to a new tube for protein recovery later by acetone precipitation or dialysis; otherwise decant to discard the supernatant.

5. Fill up the tube containing the DNA pellet with 70% ethanol from a squirt bottle, and then decant to discard the ethanol solution. Repeat the ethanol wash once. Make sure to rinse the entire interior of the tube, including the cap. Tap the tube on a clean paper towel to remove residual ethanol, leave it upside down on the paper towel for 10-15 minutes to air dry the DNA pellet.

6. Add 25 µl of TE buffer or deionized water to the 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.

7. To recover the proteins, add 4 vol (~6.4 ml) of acetone to 1 vol (~1.6 ml) of isopropanol supernatant saved at Step 4 above. Vortex and centrifuge at 12,000xg for 5 min to pellet the proteins. Decant to discard the acetone supernatant into a waste container in a chemical hood. Add 100 µl of ProMelt (#1150) to the protein pellet, scratch the tube on a tube rack and vortex to solubilize the proteins. Store the protein solution 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. 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.

2. 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. 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) 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. 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.

5. Centrifuge for 10 min at 8,000-12,000 xg to pellet the DNA/RNA. Decant to discard the entire supernatant. Gently shot 70% ethanol on the sidewall of the tube without disturbing the DNA/RNA pellet to fill up the tube. Decant to discard the ethanol solution. Put the tube upside down on a paper towel for a few minutes and then flip the tube forcefully a few times to remove residual ethanol. Air-dry the DNA/RNA pellet for 10-20 min.

6. Add 350 µl Qiagen's Buffer BM1 and vortex until the pellet is visibly dissolved. And then continue with the QIAcube protocol.

# 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. 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.

**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, 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.

**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 (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 0.5 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.