

# AquaPreserve Instruction Manual

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## General Information

### Description

AquaPreserve™ is an aqueous solution based preservative with DNA, RNA, and protein extraction functionality. It may be used to streamline blood sample collection, stabilization, transport, storage, and DNA, RNA, and protein extraction. The extraction protocols are simple. There is no need to remove the plasma or lyse the RBC or fractionate the blood cells prior to extraction. Cell-free DNA, RNA, and proteins will not be lost and gene expression profiles will not be altered artificially by in vitro separation of blood components and cells. Most uniquely, AquaPreserve can be used to extract intact total RNA from existing frozen blood samples collected in regular anticoagulants, thus enabling the use of surplus and archived frozen blood samples collected during routine medical care and clinical trials for blood RNA research.

### Specification

<b>Product Name</b>	AquaPreserve™ Kit
<b>Product #</b>	8001, 8060
<b>Size</b>	8001: 1 ml; 8060: 60 ml (sufficient for 240 mini, 30 midi, 15 maxi preps)
<b>Kit Contents</b>	8001: 1 ml AquaPreserve Solution, User Manual 8060: 60 ml AquaPreserve Solution, User Manual
<b>MSDS</b>	Available at <a href="http://www.aquaplasmid.com">www.aquaplasmid.com</a>
<b>Storage</b>	Store tightly capped at 4 °C. Vortex the reagent to mix well before dispensing.
<b>Note</b>	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

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## AquaPreserve Blood DNA/RNA Mini Protocol

This miniprep 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. Invert to mix the AquaPreserve solution well before dispensing.

**1. Lyse the blood cells.** Add 0.25 ml of AquaPreserve (*Pre-warm the AquaPreserve solution to 22-37 °C before adding to the frozen blood sample.*) to 0.25 ml of fresh or frozen whole blood in a 1.5-ml microfuge tube. Vortex or shake to mix well or until the frozen blood sample is completely thawed in AquaPreserve (*Do not thaw the frozen blood without mixing with AquaPreserve or the RNA will be degraded during blood thawing. However, if you are only interested in blood DNA extraction, the blood sample may be thawed, aliquoted and then mixed with AquaPreserve.*).

**2. Pellet the proteins.** Add 0.125 ml of ProSink to the AquaPreserve lysed blood. Shake and/or vortex for 1-2 min to mix well and incubate at 22 °C for >30 min (*Blood DNA is now stable at 4-22 °C for a year, and blood RNA is stable at -80 °C forever, 4 °C for months, 22 °C for 2 weeks, and 37 °C for 2 days. The Blood\_AquaPreserve\_ProSink samples may be shipped at ambient temperature; however, to avoid exposing the samples to extreme ambient temperatures in the hot summer days, it is preferable to ship them on wet ice packs.*). Centrifuge at 14,000 xg for 10 min to pellet the proteins.

**3. Pellet the DNA/RNA.** Transfer the supernatant (~0.5 ml) to a new 1.5-ml microfuge tube. Add 0.8 vol (~0.4 ml) of isopropanol. Shake or vortex for 30-60 sec to mix well. Centrifuge at 14,000 xg for 10 min to pellet the DNA/RNA.

**4. Rinse the DNA/RNA pellet.** Decant to discard the supernatant. Gently shoot 50% isopropanol (or 70% ethanol) from a squirt bottle to fill up the tube (*Do not to 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. Otherwise centrifuge it again.*). 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 to solubilize the DNA and RNA, and store it at -20 °C.

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

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

## AquaPreserve Blood DNA/RNA Maxi Protocol

This maxiprep protocol uses 4 ml AquaPreserve (#8060) and 2 ml ProSink (#9030, ordered separately) to extract DNA (~200 µg) and RNA (~4 µg) from 4 ml fresh or frozen human blood collected in regular anticoagulants. The extraction may be carried out using two 15-ml conical tubes and a centrifuge with a centrifuge force >3,500 xg on a swing rotor. Invert to mix the AquaPreserve solution well before dispensing.

**1. Lyse the blood cells.** Add 4 ml of AquaPreserve (*Pre-warm the AquaPreserve solution to 22-37 °C before adding to the frozen blood sample.*) to 4 ml of fresh or frozen whole blood in a 15-ml conical tube. Invert, vortex, and shake to mix well or until the frozen blood sample is completely thawed (*Holding the tube in your palm to warm it up while shaking the tube may facilitate the penetration of AquaPreserve solution into the frozen blood sample. Do not thaw the frozen blood without mixing with AquaPreserve or the RNA will be degraded during blood thawing. However, if you are only interested in blood DNA extraction, the blood sample may be thawed, aliquoted and then mixed with AquaPreserve.*).

**2. Pellet the proteins.** Add 2 ml of ProSink to the AquaPreserve lysed blood. Shake and vortex for 1-2 min to mix well and incubate at 22 °C for >30 min (*Blood DNA is now stable at 4-22 °C for a year, and blood RNA is stable at -80 °C forever, 4 °C for months, 22 °C for 2 weeks, and 37 °C for 2 days. The Blood\_AquaPreserve\_ProSink samples may be shipped at ambient temperature; however, to avoid exposing the samples to extreme ambient temperatures in the hot summer days, it is preferable to ship them on wet ice packs.*). Centrifuge at maximal speed (>3,500 xg) for 10 min to pellet the proteins.

**3. Pellet the DNA/RNA.** Pour the supernatant (~8 ml) to a new 15-ml conical tube. Add 0.8 vol (~6.4 ml) of isopropanol. Invert and vortex for 30-60 sec to mix well. Centrifuge at maximal speed (>3,500 xg) for 10 min to pellet the DNA/RNA.

**4. Rinse the DNA/RNA pellet.** Decant to discard the supernatant. Gently shoot ~10 ml of 50% isopropanol (or 70% ethanol) from a squirt bottle to the tube, rotate to rinse the entire tube, and then decant to discard the alcohol solution (*Make sure the DNA/RNA pellet remains at the bottom of the tube before pouring off the alcohol solution.*). Repeat the alcohol rinse once. Leave the tube upside down on a paper towel to air dry the DNA/RNA pellet for 5-10 min.

**5. Solubilize the DNA/RNA pellet.** Add 1 ml deionized water to the DNA/RNA pellet. Vortex or pipette up and down to solubilize the DNA and RNA. Transfer the DNA/RNA solution to a new 1.5-ml microfuge tube (*If the DNA/RNA solution is cloudy, you may centrifuge it at 14,000 xg for 5 min to pellet any insoluble materials and transfer the clear DNA/RNA solution to a new microfuge tube.*). Store DNA/RNA solution at -20 °C.

## AquaPreserve Blood Protein Extraction Protocol

Total blood proteins may be extracted from fresh or frozen whole blood sample using the protocol below. For protein extraction along with DNA/RNA extraction from the same blood samples, simply take a portion of the AquaPreserve-lysed blood sample (*Make sure to take an aliquot of the AquaPreserve-lysed blood for protein extraction prior to ProSink addition, because proteins will no longer be solubilized after being treated with ProSink.*) to be used for DNA/RNA extraction and proceed to Step 2 below “Pellet the DNA/RNA.” Invert to mix the AquaPreserve solution well before dispensing.

**1. Lyse the blood cells.** Add 0.2 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 or until the frozen blood sample is completely thawed.

**2. Pellet the DNA/RNA.** Add 0.7 vol (0.21 ml) of isopropanol, vortex for 60 sec, and centrifuge at 3,500-14,000 xg for 5 min to pellet the DNA and RNA.

**3. Pellet the proteins.** Transfer the protein-containing supernatant (0.35 ml) to a new 2-ml microfuge tube. Add 4 vol (1.4 ml) of acetone, vortex for 60 sec, and centrifuge at 3,500-14,000 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 (#1115, order separately) 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 3,500 to 14,000 xg for 5 min to pellet any insoluble materials. Transfer the protein solution to a new microfuge tube and store at 4 or –20 °C (*Some SDS may precipitate out at these temperatures, however, it will not interfere with SDS-PAGE. Alternatively it may be re-solubilized by incubating at 65 °C for 10 min.*).

## AquaPreserve Blood Collection and Storage Protocol

There are several advantages of using AquaPreserve to stabilize blood samples for later DNA and RNA extraction. First, AquaPreserve is compatible with anticoagulated blood samples collected in routine medical care and clinical trials. The blood samples can be used for other clinical tests and only an aliquot of the extra or leftover blood samples are saved in AquaPreserve for DNA and RNA extraction. Secondly, AquaPreserve-stabilized blood sample can be stored long-term at -80 °C without risking RNA degradation due to accidental blood thawing during storage. It can be repeatedly thawed to give out aliquots for distribution or experiments. Finally, AquaPreserve not only stabilizes but also extracts DNA, RNA, and proteins without using additional extraction kits. It streamlines blood collection, transport, storage, and DNA/RNA/protein extraction, reducing pre-analytical variation and processing cost.

**1. Stabilize the blood sample.** Draw blood by standard venipuncture using a vacutainer containing anticoagulant (not fluoride/oxalate). Immediately (or as soon as feasible because some RNA transcripts may continue to change *ex vivo*) transfer an aliquot of the blood (e.g., 0.25, 0.5, 2, or 4 ml, depending on your need and sample availability) to a tube containing an equal volume of AquaPreserve, and mix well to instantly lyse the blood cells and stabilize the DNA and RNA. If the blood sample will not be used for protein extraction, add 0.5 vol of ProSink (e.g., for 1 ml AquaPreserve, use 0.5 ml ProSink) to the AquaPreserve-lysed blood, shake or vortex to mix well. After mixing the AquaPreserve-lysed blood with ProSink, DNA is stable at 22 °C for months and RNA is stable for two weeks.

**2. Transport the blood sample.** The AquaPreserve and ProSink stabilized blood samples may be shipped at mild ambient temperature to the laboratory, if the blood samples are collected in the field without a freezer or refrigerator. However, during the hot summer days, the samples may be exposed to extremely high temperature during transportation, which may cause RNA degradation. To ensure RNA integrity, the samples are best shipped overnight in insulated containers with wet ice gel packs.

**3. Store the blood sample.** Upon arrival at the laboratory, the AquaPreserve and ProSink stabilized blood samples may be stored at 22 °C for a few days or at 4 °C for a few weeks before RNA extraction. For long-term storage, the samples should be stored at -80 °C. Do not store the samples at -20 °C as the RNA yield is decreased for unknown reasons. The AquaPreserve and ProSink stabilized blood sample can be thawed, centrifuged, and take out aliquots of the clear lysate for distributing to other investigators or for use in experiments. The remaining sample can be re-frozen for later use.

**4. Extract the DNA and RNA.** At the time of DNA/RNA extraction, simply thaw the AquaPreserve and ProSink stabilized blood sample, centrifuge to pellet the proteins and recover the clear lysate, and then precipitate the DNA and RNA with 0.8 vol of isopropanol. The recovered DNA/RNA may be used directly in most DNA applications without removing the RNA (~1% of total DNA/RNA). For RNA applications, the DNA/RNA preparation should be treated with DNase I to remove the DNA.

## 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 4 °C for 12 months. Invert to mix the reagent well before dispensing.

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

ProMelt (Item # 1115) is an ancillary reagent for solubilizing protein pellet precipitated by acetone. It is not needed, if you will not recover the blood proteins. ProSink (Item # 9030) is a protein precipitating solution for DNA/RNA extraction from blood or other nuclease-rich animal tissues. ProSink is optional, if you extract DNA and RNA from bacteria, cultured cells, or plant tissues.

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

You could measure 1 ml AquaPreserve to a 15-ml conical tube, then transfer 0.4 ml AquaPreserve to the frozen blood sample, vortex to partially thaw the blood, and quickly transfer the thawed blood back to the conical tube. Repeat until the remaining blood sample is completely thawed.

### 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. However, if you add some salts (e.g., 30 mM NaOAc, pH unadjusted) to the loading dye, you should get a good separation of the 5S, 18S, 28S rRNA, and the genomic DNA bands. Alternatively you can 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 units of DNase I to 10-20 µl of DNA/RNA solution in 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 and the RNA bands are discrete. To inactivate the DNase I, use Ambion's DNase removal reagent or heat-inactivate the DNase I at 65 °C for 15 min.

### 6. Can I use AquaPreserve to extract total RNA from mouse blood?

Yes, mouse blood contains an abundance of RNA. The RNA yield is only 1-2 µg/ml of human blood, but for mouse blood, RNA yield can be 50-80 µg/ml of blood.

### 7. What's your suggestion for long-term storage of blood samples?

The best way to store a fresh blood sample for later DNA/RNA extraction is to mix 1 vol of blood with 1 vol of AquaPreserve and then 0.5 vol of ProSink, and store it at -80 °C. When it is time to extract the DNA/RNA, you simply thaw the sample, centrifuge to recover the clear lysate, and use ISOH to precipitate the DNA/RNA. You don't need to worry freezer malfunction, you can freeze-thaw and take aliquots during storage, and you don't need to use other kits for the extraction.