AquaPure Genomic DNA Kits

Catalog

732-6340	Genomic DNA Kit
732-6343	Genomic Tissue Kit
732-6345	Genomic DNA Blood Kit

BIO-RAD

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Section 1
Introduction to Quantum Prep AquaPure Genomic DNA Kits

1.1 Overview

The AquaPure Genomic DNA Kits come in three varieties. Each is specifically designed for isolating DNA from cells, cells and tissue, or cells and blood. The kits come complete with all the solution components and procedures necessary for isolating genomic DNA from sample sources such as whole blood and bone marrow, cultured cells, animal and plant tissues, and gram-negative bacteria. For other procedures and additional information, contact Bio-Rad Laboratories' Technical Services.

DNA is isolated from cells such as cultured mammalian cells, white blood cells, animal tissue, or microbes by first lysing the cells with an anionic detergent in the presence of a DNA stabilizer. Most mammalian whole blood and bone marrow specimens contain both non-nucleated cells (red blood cells) and nucleated cells (white blood cells) which contain DNA. When isolating DNA from whole blood or bone marrow specimens, the red blood cells, which lack genomic DNA, are first lysed to facilitate their separation from the white blood cells. The DNA stabilizer in the Lysis Buffer works by limiting the activity of DNases that are contained in the cell and elsewhere in the environment. Contaminating RNA is then removed by treatment with an RNA-digesting enzyme. Other contaminants, such as proteins, are removed by salt precipitation. Finally, the genomic DNA is recovered by precipitation with alcohol and dissolved in a buffered solution containing the DNA stabilizer.

Purified genomic DNA is required in DNA analysis procedures including DNA amplification, Southern and dot/slot blot hybridization, sequencing, and cloning. Isolation of genomic DNA using salt as a substitute for toxic organic solvents in the deproteination step has been described. The AquaPure Genomic DNA Kit uses procedures modified from these methods to rapidly isolate high quality DNA suitable for DNA analysis.

1.2 Quality Assurance

All components of the AquaPure Genomic DNA Isolation Kits are subjected to a rigorous, two-stage quality assurance testing process:

- Component Testing: Each kit component is tested to insure the absence of DNase, DNA and microbial contamination.
- Performance Testing: Each AquaPure Kit is tested for the ability to isolate DNA that meets performance specifications for yield and quality. Performance testing quality specifications for isolated DNA include the absence of protein contamination, restriction enzyme digestibility and ability to amplify in a PCR-based assay.

These measures help to ensure good results. If poor results occur, please contact Bio-Rad Laboratories' Technical Services.

1.3 Kit Components

The AquaPure Genomic Isolation kit (catalog number 732-6340) contains materials and reagents sufficient for 100 preps.

35 ml	Genomic DNA Lysis Solution
12 ml	Protein Precipitation Solution
10 ml	DNA Hydration Solution
250 µl	RNase A Solution (4 mg/ml)

AquaPure Genomic Blood kit (catalog number 732-6345) contains materials and reagents sufficient for 100 preps.

100 ml	RBC Lysis Solution
35 ml	Genomic DNA Lysis Solution
12 ml	Protein Precipitation Solution
10 ml	DNA Hydration Solution
250 µl	RNase A Solution (4 mg/ml)

AquaPure Genomic DNA Tissue Kit (catalog number 732-6343) contains materials and reagents sufficient for 100 preps.

35 ml	Genomic DNA Lysis Solution
12 ml	Protein Precipitation Solution
10 ml	DNA Hydration Solution
250 µl	RNase A Solution (4 mg/ml)
175 µl	Proteinase K Solution (20 mg/ml)

1.4 Materials Required but Not Supplied

Reagents:	100% Isopropanol
	70% ethanol
	Liquid nitrogen (for tissues)
	Anti-coagulants (EDTA, heparin, etc.)
Equipment:	Cell scrapers (for tissue culture cells)
	Mortar and pestle (for tissues)
	Microfuge pestle (for tissues)

1.5 Storage and Stability

Store the Proteinase K Solution at 4 °C. Store all other AquaPure reagents at room temperature, including RNase A Solution. All components are guaranteed for 12 months from the date of purchase, when stored at room temperature and used as described in this manual.

Section 2
Experimental Procedures

2.1 Recommendations for Best Results

DNA Hydration

It is recommended for best results that DNA hydration involve an overnight incubation. Please follow the following steps if best results are required:

1. After adding the appropriate amount of DNA Hydration Solution, rehydrate DNA by incubating at 65 °C for 1 hour or overnight at room temperature. If possible, tap tube periodically to aid in dispersing the DNA.
2. Store DNA at 4 °C. For long term storage, store at -20 °C or -80 °C.

2.2 Helpful Hints

If high molecular weight DNA is required follow these guidelines:

1. When collecting tissue samples, place fresh tissue directly into Genomic DNA Lysis Solution. Homogenizing or freezing tissue immediately upon collection will minimize DNase activity and result in increased quality and size of DNA.
2. In case of incomplete cell lysis due to clumping of cells, treatment with Proteinase K can be helpful. In the standard protocol, 1.5 µl of the Proteinase K stock solution (20 mg/ml) added to 300 µl Genomic DNA Lysis Solution gives a final concentration of 100 µg/ml. Incubate cells at 55 °C in 100 µg/ml Proteinase K for 1 hr to overnight until cells are completely lysed.

3. When storing the isolated DNA, use methods that help to preserve its integrity. For long-term sample storage (>5 days), store samples at -80 °C, or, alternatively, in Genomic DNA Lysis Solution at room temperature. For short-term sample storage (<5 days), store samples at 4 °C or, alternatively, in Genomic DNA Lysis Solution at room temperature.

Measuring DNA purity and quality:

When isolating DNA using the AquaPure Genomic DNA Isolation Kit, the expected A_{260}/A_{280} ratio ranges from 1.7–2.0. Ratios lower than this may indicate protein contamination. However, DNA sample ratios lower than 1.4 can be amplified in most cases without problems. Ratios above 2.0 may indicate RNA contamination. Increasing RNase incubation time in cell/tissue lysate from 15 minutes to 30-60 minutes may be necessary to remove more of the contaminating RNA. To remove contaminating RNA from a purified sample, add the same volume of RNase A Solution to the DNA sample as used during the initial DNA isolation procedure. Then incubate at 37° for 15–60 minutes. Following the incubation, remove digested RNA and RNase A by re-precipitating the DNA as indicated in the Appendix, Section 5.1.

DNA quality can also be assessed by analyzing the DNA via agarose gel electrophoresis or by evaluating performance (*e.g.*, by PCR amplification or restriction enzyme digest analysis).

Section 3 Protocols

Important Note: Protein Precipitation, DNA Precipitation and DNA Hydration steps are identical for all isolation protocols and are found in **Section 3.7.**

3.1 Protocol for Extraction of DNA from Cultured Cells

Sample Collection and Handling

1. Add 1–2 million cells in balanced salt solution or culture medium to a 1.5 ml microfuge tube.
2. Centrifuge at 13,000–16,000 x g for 5 seconds to pellet cells. Remove supernatant leaving behind 10–20 µl residual liquid.
3. Vortex the tube vigorously to resuspend the cells in the residual supernatant. This greatly facilitates cell lysis in Step 4 below.
4. Add 300 µl Genomic DNA Lysis Solution to the resuspended cells and carefully pipet up and down several times to lyse the cells, making sure not to shear the genomic DNA. Usually no incubation is required; however, if cell clumps are visible after mixing, incubate at 37 °C until the solution is homogeneous. Samples are stable in Genomic DNA Lysis Solution for at least 18 months at room temperature.

RNase Treatment

1. Add 1.5 µl RNase A Solution (4 mg/ml) to the cell lysate.
2. Mix the sample by inverting the tube 25 times and incubate at 37 °C for 5 minutes.
   Note: Incubation may be performed for up to 60 minutes.
3. Go to Section 3.7, Protein Precipitation, DNA Precipitation, and DNA Hydration Protocol.

3.2 Protocol for Extraction of DNA from Tissue

Sample Collection and Handling

1. Samples may be fresh, frozen, or fixed.
2. Dissect tissue sample quickly and freeze in liquid nitrogen. Store at -70° to -80 °C.
3. Prepare tissue sample for DNA isolation by grinding in liquid nitrogen with a mortar and pestle.
4. If fresh tissue is being used, work very quickly and keep tissue on ice at all times, including when tissue is being weighed.

DNA Isolation From 5–10 mg Fresh or Frozen Solid Tissue
Expected Yield: 2–15 µg DNA

Cell Lysis

1. Add 5–10 mg of frozen or fresh ground tissue of to a 1.5 ml microfuge tube containing 300 µl Genomic DNA Lysis Solution and homogenize thoroughly using a microfuge tube pestle. Place sample on ice until next step.
2. Choose either procedure A or B (If maximum yield is required we suggest using B):
    A. Incubate lysate at 65 °C for 15–60 minutes. We suggest incubating 45 minutes at 65 °C for best results. Invert tube periodically during the incubation.
    B. Add 1.5 µl Proteinase K Solution (20 mg/ml) to the lysate. Mix by inverting 25 times and incubate at 55 °C for 2–3 hours (to overnight) or until tissue particulates have dissolved. If possible, invert tube periodically during the incubation.

RNase Treatment

1. Add 1.5 µl RNase A Solution (4 mg/ml) to the cell lysate.
2. Mix the sample by inverting 25 times, and incubate at 37 °C for 15–60 minutes. We suggest incubating 45 minutes at 65 °C for best results.
3. Go to Section 3.7, Protein Precipitation, DNA Precipitation, and DNA Hydration Protocol.

3.3 Protocol for Extraction of DNA from Mouse Tail

DNA Isolation From 5 mm (5–10 mg) Mouse Tail Tissue
Expected Yield: 10–75 µg DNA

Cell Lysis

1. Place 5 mm (5–10 mg) fresh or frozen mouse tail tissue (minced if possible) into a 1.5 ml microfuge tube containing 300 µl Genomic DNA Lysis Solution.
2. Add 1.5 µl Proteinase K Solution (20 mg/ml) to the lysate and mix by inverting 25 times. Incubate at 55 °C overnight (or until tissue has dissolved). If possible, invert tube periodically during the incubation. Note: Undigested invertebrae and hair will be visible in the tube.

RNase Treatment

1. Add 1.5 µl RNase A Solution (4 mg/ml) to the cell lysate.
2. Mix sample by inverting the tube 25 times and incubate at 37 °C for 15-60 minutes.

3. Go to Section 3.7, Protein Precipitation, DNA Precipitation, and DNA Hydration Protocol.

3.4 Protocol for Extraction of DNA from Blood

A. Standard DNA Isolation From 300 µl Whole Blood

Standard 45 Minute Protocol, Includes RNase Treatment
Expected Yield: 5–15 µg DNA

Sample Collection and Handling Whole Blood

1. If possible, collect whole blood and bone marrow in EDTA to reduce DNA degradation. However, other anticoagulants such as ACD (citrate) and heparin may also be used successfully.
2. Fresh samples can be stored at 4 °C for as long as 5 days.
3. Frozen samples are stable at -80 °C for at least two years. Before use, thaw quickly in a 37 °C water bath and keep sample on ice until use.

Cell Lysis

1. Add 300 µl whole blood (or bone marrow) to a 1.5 ml microfuge tube containing 900 µl RBC Lysis Solution. Invert to mix and incubate 10 minutes at room temperature; invert again at least once during the incubation.
2. Centrifuge for 20 seconds at 13,000–16,000 x g. Remove supernatant with a pipette leaving behind the visible white cell pellet and about 10–20 µl of the residual liquid.
3. Vortex the tube vigorously to resuspend the cells in the residual liquid; this greatly facilitates cell lysis in Step 4 below.

4. Add 300 µl Genomic DNA Lysis Solution to the resuspended cells and carefully pipet up and down several times to lyse the cells, making sure not to shear the genomic DNA. Usually no incubation is required. However, if cell clumps are visible after mixing, incubate at 37 °C or room temperature until the solution is homogeneous. 30 minutes at 37 °C with occasional inversion is usually sufficient for homogeneity. Samples are stable in Genomic DNA Lysis Solution for at least 18 months at room temperature.

RNase Treatment (Optional)

1. Add 1.5 µl RNase A Solution (4 mg/ml) to the cell lysate.
2. Mix the sample by inverting the tube 25 times and incubate at 37 °C for 15–60 minutes.
3. Go to Section 3.7, Protein Precipitation, DNA Precipitation, and DNA Hydration Protocol.

B. Rapid DNA Isolation From 300 µl Whole Blood

Rapid 25 Minute Protocol, Does Not Include RNase Treatment
Expected Yield: 5–15 µg DNA

Sample Collection and Handling Whole Blood

1. If possible, collect whole blood and bone marrow in EDTA to reduce DNA degradation. However, other anticoagulants such as ACD (citrate) and heparin may also be used successfully.
2. Fresh samples can be stored at 4 °C for no more than 5 days.
3. Frozen samples are stable at -80 °C for at least two years. Before use, thaw quickly in a 37°C water bath and keep sample on ice until use.

Cell Lysis

1. Add 300 µl whole blood (or bone marrow) to a 1.5 ml microfuge tube containing 900 µl RBC Lysis Solution. Incubate 1 minute at room temperature; invert gently 10 times during the incubation. *Note: For fresh blood, collected within 1 hour, increase incubation time to 3 minutes to ensure complete red blood cell lysis.*
2. Centrifuge for 20 seconds at 13,000–16,000 x g. Remove as much supernatant as possible with a pipette leaving behind the visible white cell pellet and about 10–20 µl of the residual liquid.
3. Vortex the tube vigorously for 10 seconds to resuspend the white cells in the residual liquid; this greatly facilitates cell lysis in Step 4 below. The white cell pellet should not be visible following vortexing.
4. Add 300 µl Genomic DNA Lysis Solution to the resuspended cells and carefully pipet up and down to lyse the cells, making sure not to shear the genomic DNA. Samples are stable in Genomic DNA Lysis Solution for at least 18 months at room temperature.
5. Go to Section 3.7, Protein Precipitation, DNA Precipitation, and DNA Hydration Protocol.

3.5 Protocol for Extraction of DNA from Buffy Coat

DNA Isolation from Buffy Coat Prepared from 300 µl Whole Blood

Expected Yield: 5–15 µg DNA

Sample Collection and Handling Whole Blood

1. If possible, collect whole blood and bone marrow in EDTA to reduce DNA degradation. However, other anticoagulants such as ACD (citrate) and heparin may also be used successfully.
2. Store fresh samples at 4 °C for not longer than 5 days.
3. Frozen samples are stable at -80 °C for at least two years. Before use, thaw quickly in a 37 °C water-bath and keep sample on ice until use.

Buffy Coat

1. If possible, collect whole blood in EDTA to reduce DNA degradation. Other anticoagulants such as ACD (citrate) and heparin may be used successfully.
2. Store fresh samples at 4 °C.
3. Isolate white blood cells using AquaPure RBC Lysis Solution (see Cell Lysis segment of Section 3.4).
4. Alternatively to the RBC Lysis procedure, a buffy coat can be prepared by centrifuging whole blood sample at 800 x g for 10 minutes at room temperature. Or place tube containing blood in a vertical position at 4 °C overnight to allow cells to settle. A thin layer of white blood cells (buffy coat) should be visible between the upper plasma layer and the lower red blood cell layer. Remove the upper plasma layer and then carefully collect the buffy coat with a pipet. Keep sample on ice until use.

Cell Lysis

If the buffy coat preparation contains red blood cells, treat sample with RBC Lysis Solution by beginning with Step 1 below. If buffy coat preparation is clean and free of red blood cells, pipet

15–25 µl sample into a 1.5 ml microfuge tube containing 300 µl Genomic DNA Lysis Solution as instructed in Step 4 below.

1. Add 15–25 µl buffy coat prepared from a 300 µl whole blood sample to a 1.5 ml microfuge tube containing 3 parts RBC Lysis Solution (for example, mix 25 µl buffy coat sample with 75 µl RBC Lysis Solution). Invert to mix and incubate 5 minutes at room temperature; invert again at least once during the incubation.
2. Centrifuge for 20 seconds at 13,000–16,000 x g. Remove supernatant with a pipette leaving behind the visible white cell pellet and about 10-20 µl of the residual liquid.
3. Vortex the tube vigorously to resuspend the cells in the residual liquid; this greatly facilitates cell lysis in Step 4 below.
4. Add 300 µl Genomic DNA Lysis Solution to the resuspended cells and pipet up and down to lyse the cells. Usually no incubation is required; however, if cell clumps are visible after mixing, incubate at 37 °C or room temperature until the solution is homogeneous. Samples are stable in Genomic DNA Lysis Solution for at least 18 months at room temperature.

RNase Treatment (Optional)

1. Add 1.5 µl RNase A Solution (4 mg/ml) to the cell lysate.
2. Mix the sample by inverting the tube 25 times and incubate at 37 °C for 15–60 minutes.
3. Go to Section 3.7, Protein Precipitation, DNA Precipitation, and DNA Hydration Protocol.

3.6 Protocol for Extraction of DNA from Gram-Negative Bacteria

Sample Collection and Handling

1. Samples may be either fresh or frozen.
2. Collect overnight bacterial cultures and store on ice until use.

DNA Isolation From 0.5 ml Gram-Negative Bacterial Culture
Expected Yield: 10–35 µg DNA

Cell Lysis

1. Add 500 µl cell suspension (*e.g.*, overnight culture containing 0.5–1.5x10^9 cells) to a 1.5 ml microfuge tube on ice.
2. Centrifuge at 13,000–16,000 x g for 15 seconds to pellet cells. Carefully remove as much supernatant as possible with a pipet.
3. Add 300 µl Genomic DNA Lysis Solution and pipet up and down until cells are suspended.
4. Incubate sample at 80 °C for 5 minutes to lyse cells. Samples are stable in Genomic DNA Lysis Solution for at least 18 months at room temperature.

RNase Treatment

1. Add 1.5 µl RNase A Solution (4 mg/ml) to the cell lysate.
2. Mix the sample by inverting the tube 25 times and incubate at 37 °C for 15–60 minutes. We suggest 45 minutes at 37 °C for best results.
3. Go to Section 3.7, Protein Precipitation, DNA Precipitation, and DNA Hydration Protocol.

3.7 Protein Precipitation, DNA Precipitation and DNA Hydration Protocol

Important Note: The following Protein Precipitation, DNA Precipitation and DNA Hydration steps are used for all the protocols in the above sections.

Protein Precipitation

1. If an RNase Step was performed, cool the sample to room temperature before adding the Protein Precipitation Buffer in step 2.
2. Add 100 µl Protein Precipitation Solution to the cell lysate.
3. Vortex vigorously at high speed for 20 seconds to mix the Protein Precipitation Solution uniformly with the cell lysate.
4. Centrifuge at 13,000–16,000 x g for 3 minutes. The precipitated proteins should form a tight pellet of cellular debris. If the pellet is not tight, repeat Step 3, followed by incubation on ice for 5 minutes and then repeat Step 4.

DNA Precipitation

1. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 1.5 ml microfuge tube containing 300 µl 100% isopropanol (2-propanol). If the DNA yield is expected to be <1 µg, add a DNA carrier such as glycogen (recommendation: 0.5 µl of 20 mg/ml glycogen per 300 µl of isopropanol).
2. Mix the sample by inverting gently 50 times.
3. Centrifuge at 13,000–16,000 x g for 1 minute; the DNA will be visible as a small white pellet.

4. Pour off supernatant and drain tube briefly on clean absorbent paper. Add 300 µl 70% ethanol and invert the tube several times to wash the DNA pellet.
5. Centrifuge at 13,000–16,000 x g for 1 minute. *Carefully pour off the ethanol. Pellet may be loose so pour slowly and watch pellet.*
6. Invert and drain the tube on clean absorbent paper and allow to air dry 10–15 minutes.

DNA Hydration

1. Add 50–100 µl of DNA Hydration Solution (for the 300 µl Blood Protocol we recommend that you hydrate your DNA in 100 µl DNA Hydration Buffer).
2. Incubate sample at 65 °C for 5 minutes (up to 1 hr.) to accelerate hydration. Alternatively, incubate sample overnight at room temperature.
3. Vortex 5 seconds at medium speed to mix. Pulse spin briefly to collect sample at bottom of the tube.
4. Store DNA at 4 °C. For long term storage, store at -20 °C or -80 °C.

Section 4
Troubleshooting

Possible Cause/Problem	Solutions or Suggestions
RBC Lysis Step of the Procedure	
The red blood cells in the sample were not completely lysed with RBC Lysis Buffer	• There was a higher than average number of red blood cells in the sample. Repeat the incubation with RBC Lysis Buffer to lyse the remaining red blood cells. Add 3 parts RBC Lysis Buffer for every 1 part of sample, incubate 10 minutes at room temperature then centrifuge according to the original protocol followed.
Blood clots are present in the whole blood sample	• The sample was not mixed or stored properly during blood collection. The sample may be handled as follows: A. Isolate DNA from the unclotted portion of the sample only; *i.e.*, when blood is removed from the collection tube, leave clots behind and isolate DNA from unclotted portion. B. Remove large clots from white blood cell pellet. To facilitate clot removal, resuspend cells in phosphate buffered saline and remove the clots with either forceps or pipet tip. After removing the clot, centrifuge to pellet the white cells, discard the supernatant and proceed with the isolation protocol. C. Remove small clots by digestion with Proteinase K. Add Proteinase K Solution (20 mg/ml) to the Genomic DNA Lysis Solution to a final concentration of 100 µg/ml and incubate at 55 °C with periodic mixing until the clots are lysed completely. *Note: It is very important that blood clots be lysed completely before proceeding to the Protein Precipitation step of the procedure to ensure maximum DNA yield with minimum protein contamination.*

Possible Cause/Problem	Solutions or Suggestions
DNA Yield	
Low DNA Yield	• There were an insufficient number of cells in the starting sample material. Count cells or weigh tissue prior to beginning the Cell Lysis step. It is important to use the specified amount of starting material for a given protocol. Too few cells will result in a lower DNA concentration during the DNA Precipitation Step and will reduce DNA precipitation efficiency. The result is a low DNA yield. Adding a carrier, such as glycogen, will help to maximize DNA yield. We recommend adding 0.5 µl glycogen (20 mg/ml) per 300 µl 100% isopropanol. • Cells were not completely lysed due to adding too many cells or too much tissue to the Genomic DNA Lysis Solution. Count cells or weigh tissue prior to beginning the Cell Lysis step. Too many cells may overload the chemistry inhibiting complete cell lysis, which results in a low DNA yield. • There were cell clumps present in the sample after adding Genomic DNA Lysis Solution. Cell clumps may occur when cells are not completely resuspended prior to addition of Genomic DNA Lysis Solution. To lyse the cells in the clumps, incubate sample at either 37 °C or room temperature with periodic mixing until the solution is homogeneous. Cell clumps may be dissolved more quickly by adding Proteinase K Solution (20 mg/ml) to a final concentration of 100 µg/ml and incubating at 55 °C until cells are completely lysed (1 hour to overnight). • If fewer than 200,000 cells per 300 µl Genomic DNA Lysis Solution are used for DNA isolation or if the DNA yields is expected to be low (<1 µg), add a DNA carrier such as glycogen to the isopropanol. We recommend adding 0.5 µl glycogen (20 mg/ml) per 300 µl isopropanol.

Possible Cause/Problem	Solutions or Suggestions
	• Sample is not completely hydrated. Continue to hydrate sample by either incubating sample overnight at room temperature or by incubating at 65°C for 1 hour (samples may be incubated at 65°C for a total of 2 hours without affecting DNA quality).
DNA Concentration	
DNA yield was lower than expected resulting in a lower than expected DNA concentration	• Consult the Appendix, **Section 5.1**, to concentrate the DNA by precipitation.
Cell Lysis Step of the Procedure	
Cells are incompletely lysed	• There were too many cells for the amount of Genomic DNA Lysis Solution used, which overloaded the system. The presence of too many cells will inhibit cell lysis; the Genomic DNA Lysis Solution will become very viscous and cells will clump. Add more Genomic DNA Lysis Solution to completely lysed the cells. To prevent incomplete cell lysis, either count cells with a hemacytometer or other cell counter or weigh tissue samples prior to beginning the Cell Lysis Step of the procedure.
	• Cell clumps were present after adding Genomic DNA Lysis Solution. Cell clumps may occur when cells are not completely resuspended prior to addition of Lysis Solution. To lyse the cells in the clumps, incubate sample at either 37 °C or room temperature with periodic mixing until the solution is homogeneous. Cell clumps may be dissolved more quickly by adding Proteinase K Solution (20 mg/ml) to a final concentration of 100 µg/ml and incubating at 55 °C until cells are completely lysed (1 hour to overnight).
Incomplete Cell Lysis of cells fixed in methanol:acetic acid or ethanol.	• Fixative was not adequately removed from the sample prior to adding Genomic DNA Lysis Solution. Remove fixative completely after centrifuging to pellet the cells; do not wash cells with PBS as this will result in a greatly reduced yield.

Possible Cause/Problem	Solutions or Suggestions
	• Cell clumps are present after adding Genomic DNA Lysis Solution to the sample. Incubate samples at 37 °C or room temperature with periodic mixing until the solution is homogeneous. Cell clumps may be dissolved more quickly by adding Proteinase K Solution (20 mg/ml) to a final concentration of 100 µg/ml and incubating at 55 °C until cells are completely lysed (1 hour to overnight).
Incomplete lysis of cells Wash isolated from a Ficoll	• Ficoll was not adequately removed. cells once in phosphate buffered saline to remove Ficoll prior to adding Genomic DNA Lysis Solution. After adding Genomic DNA Lysis Solution, incubate the lysate at 65 °C to complete lysis if necessary.
Protein Precipitation Step of the Procedure	
No protein pellet, or pellet is soft or loose	• Sample was not cooled sufficiently (<22 °C) after the RNase digestion step and before adding Protein Precipitation Solution. To obtain a tight protein pellet, be sure that the sample is cooled to room temperature or below prior to adding Protein Precipitation Solution. To obtain a tight protein pellet: A. Re-vortex the sample for 20 seconds to mix the Protein Precipitation Solution uniformly with the cell lysate. B. Incubate sample on ice for 5–15 minutes. Incubation on ice should facilitate forming a tight pellet. C. Centrifuge according to the protocol followed to pellet the precipitated proteins. • Protein Precipitation Solution was not mixed uniformly with the cell lysate. Be sure to vortex vigorously for the full 20 seconds as specified in the protocol.

Possible Cause/Problem	Solutions or Suggestions
	• Centrifuge speed set incorrectly. Set centrifuge speed to the g-force specified in the protocol. For microfuge tube preps, set the centrifuge speed to maximum. For tabletop and other centrifuges, the speed should usually be set to 2,000 x g. If a g-force of 2,000 x g cannot be attained by your centrifuge, increase centrifugation time to achieve the same total g-force. For example, if the required g-force and time are 2,000 x g for 10 minutes, that computes to a total g-force of 20,000 x g x minutes. If the centrifuge only achieves 1,600 x g, then it will be necessary to spin at 1,600 x g for 12.5 minutes [(1,600 x g) (12.5 minutes) = 20,000 x g x minutes)]. Please note that 2,000 x g and 2,000 rpm are not equivalent.
DNA Hydration Step of Procedure	
Samples are slow to rehydrate	• Samples were not mixed during the hydration step. Mix samples periodically by gently tapping the tube to aid in dissolving the DNA.
	• The DNA pellets were over-dried prior to adding DNA Hydration Solution. DNA pellets that are dried for longer than 15 minutes at room temperature or dried using vacuum and heat will require a longer time to rehydrate completely. Hydrate DNA by incubating at 65 °C for 1 hour and then overnight at room temperature. *Note: incubation at 65 °C overnight is not recommended, as it will reduce the DNA size.*
Protein contamination in the rehydrated DNA sample	• Protein contamination usually results from exceeding the recommended amount of sample material. Follow the Re-purification of Isolated DNA, in the Appendix, **Section 5.2**.

Possible Cause/Problem	Solutions or Suggestions
DNA Quality	
A_{260}/A_{280} too high or too low	• When isolating DNA using the AquaPure Genomic DNA Isolation Kit, the expected A_{260}/A_{280} ranges from 1.7–2.0. Ratios lower than this may indicate protein contamination. However, DNA samples with ratios lower than 1.4 can be amplified in most cases without problems. DNA quality can also be assessed by analyzing the DNA via agarose gel electrophoresis or by evaluating performance (*e.g.*, by PCR amplification or restriction enzyme digest analysis). • Ratios above 2.0 may indicate RNA contamination. A. Increase RNase incubation time in cell lysate from 15 minutes to 30–60 minutes. B. To remove contaminating RNA from a purified sample, add the same volume of RNase A Solution (4 mg/ml) to the DNA sample as used during the initial DNA isolation procedure. Incubate at 37 °C for 15–60 minutes. Following the incubation, remove digested RNA and RNase A by re-precipitating the DNA as indicated in the Appendix, **Section 5.1**.
Isolated DNA is less than 50 kb in size	• DNA is degraded due to improper sample collection or storage of stating material. Collect and store samples using methods that preserve DNA integrity. For long-term sample storage (>5 days), store samples frozen at -80 °C, or, alternatively, in Genomic DNA Lysis Solution at room temperature. For short-term sample storage (<5 days), store samples at 4 °C or, alternatively, in Genomic DNA Lysis Solution at room temperature.

Possible Cause/Problem	Solutions or Suggestions
	• DNA is sheared due to over-handling (over homogenizing tissue samples) in Genomic DNA Lysis Solution or DNA Hydration Solution. For tissue samples, placing fresh tissue directly into Lysis Solution and homogenizing immediately or freezing tissue immediately upon collection will minimize DNase activity and result in increased DNA size.
	• Please note: The AquaPure method is a very gentle method that produces a minimum amount of shearing compared to organic or other methods of DNA extraction. Vortexing for 20 seconds at the Protein Precipitation Step of the procedure will not affect the size or quality of the isolated DNA.

Section 5
Appendix

5.1 Re-precipitation of DNA

If your DNA is contaminated with excess salt due to inefficient washing during the purification, re-precipitation of the DNA may be required. The following protocol is designed to remove salts and prepare the DNA for efficient downstream processing. This protocol can also be used to concentrate a sample.

1. Add Protein Precipitation Solution and 100% ethanol to DNA samples in the ratio shown in the table below. If DNA yield is expected to be low, add glycogen as a precipitating agent (see table below for volumes).
2. Invert gently 50 times and incubate at room temperature 15 minutes.

3. Centrifuge at 13,000–16,000 x g for 5 minutes (microfuge tube prep) or at 2,000 x g for 10 minutes (15 ml or 50 ml tube prep).
4. Pour off the supernatant and add 70% ethanol (see table below). Invert gently to wash the DNA.
5. Centrifuge at 13,000–16,000 x g for 1 minute (microfuge tube prep) or at 2,000 x g for 2 minutes (15 ml or 50 ml tube prep).
6. Pour off supernatant, drain on clean absorbent paper and allow DNA to air dry 10-15 minutes.
7. Add appropriate volume of DNA Hydration Solution. *Note: adding 1/5 the volume of DNA Hydration Solution used previously will increase the DNA concentration by 5 times.*
8. Store DNA at 4 °C. For long term storage, store sample at -20 °C or -80 °C.

Solution	Recommended Ratio of Solution	Volume to Add for a 100 µl DNA Sample
Protein Precipitation Solution	½ volume	50 µl
100% Ethanol	2 volumes	200 µl
Glycogen Solution (20 mg/ml)	Final concentration of 33.3 µl/ml	0.5 µl
70% Ethanol	3 volumes	300 µl

5.2 Re-purification of Isolated DNA

Please follow this procedure if you are not satisfied with the purity of your isolated DNA. This protocol is designed for DNA samples of poor quality that have either been isolated by other methods, or, from your AquaPure prep, *e.g.*, due to overloading, skipping a step or protein contamination.

1. Add 5 parts Genomic DNA Lysis Solution for every 1 part DNA Hydration Solution used to hydrate the DNA sample. Pipet up and down to mix.
2. Incubate sample at 37 °C in Genomic DNA Lysis Solution until protein particulates have dissolved. If it is difficult to dissolve the protein pellets, Proteinase K Solution (20 mg/ml) may be added to a final
   concentration of 100 µg/ml; incubate sample at 55 °C until particulates have dissolved (1 hour to overnight). To obtain maximum yield, it is important that particulates be dissolved completely before proceeding.
3. Add 2 parts Protein Precipitation Solution and vortex vigorously at high speed for 20 seconds.
4. Centrifuge to pellet the proteins; Centrifuge at 13,000–16,000 x g for 3 minutes (microfuge-tube prep) or at 2,000 x g for 10 minutes (15 ml or 50 ml tube prep)
5. Pour the supernatant into a clean tube containing 6 parts 100% isopropanol. If the DNA yield is expected to be low (<1 µg), add a DNA carrier such as glycogen according to the table below.
6. Mix by inverting gently 50 times.
7. Centrifuge to pellet the DNA; Centrifuge at 13,000–16,000 x g for 1 minute (microfuge-tube prep) or at 2,000 x g for 5 minutes (15 ml or 50 ml tube prep).
8. Pour off supernatant and wash DNA with 6 parts 70% ethanol.
9. Centrifuge at 13,000–16,000 x g for 1 minute (microfuge-tube prep) or at 2,000 x g for 1 minute (15 ml or 50 ml tube prep). Carefully pour off the ethanol.
10. Allow DNA to air dry for 10–15 minutes.

11. Add 1 part DNA Hydration Solution (or appropriate volume). Allow DNA to hydrate overnight at room temperature or at 65 °C for 1 hour.
12. Store DNA at 2–8 °C. For long term storage, store at –20 to -80 °C.

Examples

Reagents	Volume of Hydration Solution Added			
	100 µl	200 µl	500 µl	1000 µl
Genomic DNA Lysis Solution	500 µl	1 ml	2.5 ml	30 µl
Proteinase K Solution (20 mg/ml)	3 µl	6 µl	15 µl	30 µl
Protein Precipitation Solution	200 µl	400 µl	1 ml	2 ml
100% Isopropanol	600 µl	1.2 ml	3 ml	6 ml
70% Ethanol	600 µl	1.2 ml	3 ml	6 ml
DNA Hydration Solution	100 µl	200 µl	500 µl	1000 µl

5.3 High-Throughput Large Sample Number Processing Tips

The following are tips for high-throughput sample processing using the AquaPure Genomic DNA Isolation Kit. Please contact Bio-Rad Laboratories' Technical Services with any questions or for additional information.

1. Process 24 samples at time using 24-place tube racks. Process 4 racks per day = 96 samples or 5 racks/day = 120 samples per day.
2. Use dedicated centrifuges with 24-place rotors.
3. Pre-aliquot RBC Lysis Solution and 100% isopropanol on a weekly basis.

4. Use repipettors to dispense RBC Lysis Solution, Genomic DNA Lysis Solution, Protein Precipitation Solution, 100% isopropanol, 70% ethanol and DNA Hydration Solution. Using repipettors helps prevent contamination of reagents and saves time in pipetting.
5. To save time in labeling tubes, use removable labels (preferably computer generated) and transfer label from lysis tube to isopropanol tube. Or, use a computer to generate two labels, one for each tube.
6. Vortex for 10 seconds at high speed after adding Genomic DNA Lysis Solution instead of pipetting up and down to lyse the cells.
7. Omit RNase Treatment step, or, alternatively, add RNase A Solution to Genomic DNA Lysis Solution before beginning the isolation. RNase A Solution (4 mg/ml) is stable in Genomic DNA Lysis Solution for at least 1 hour at room temperature.
8. Invert tubes in rack in 100% isopropanol rather than inverting each tube individually.
9. After rehydration transfer samples isolated in 15 ml or 50 ml centrifuge tubes to 2-ml screw capped tubes (or other storage tubes) labeled with small computer labels.

5.4 Scaling Tables for Larger Size Samples

The following tables are used to facilitate scaling up the sample sizes used in the AquaPure Genomic DNA Kit protocols. Please contact Bio-Rad Laboratories' Technical Service with any questions or for detailed protocols and additional information regarding these procedures.

Cultured Cells Reagent Volumes Scaling Table (100 Cells to 50 x10^6 Cells)

Number Cells[1]	100–10,000	0.5–1 x10^6	3–5 x10^6	30–50 x10^6
Tube Size	0.6 ml	1.5 ml	1.5 ml	15 ml
Genomic DNA Lysis Solution	60 µl	150 µl	600 µl	6 ml
RNase A Solution (4 mg/ml)	0.5 µl	0.75 µl	3 µl	30 µl
Protein Precipitation Solution	20 µl	33 µl	200 µl	2 ml
100% Isopropanol[2]	60 µl	150 µl	600 µl	6 ml
DNA Hydration Solution[3]	10 µl	10 µl	60 µl	500 µl
Expected DNA Yield Range[4]	0.4–40 ng	2.0–6.0 µg	15–30 µg	80–300 µg

[1] The number of cells in a tissue culture sample may be determined by counting with a hemacytometer or other cell counter.

[2] If the DNA yield is expected to be low (for example, if the number of cells in the sample is low) add a DNA carrier such as glycogen to the isopropanol. Add 1 µl glycogen (20 mg/ml) per 600 µl 100% isopropanol.

[3] Most DNA Hydration Solution volumes give a DNA concentration of approximately 400 µg/ml. Concentration can be increased by adding less DNA Hydration Solution or decreased by adding more DNA Hydration Solution.

[4] DNA yield assumes 6 pg DNA per human diploid cell and 80% recovery.

Solid Tissue Reagent Volumes Scaling Table (0.5–200 mg Tissue)

Amount Tissue (mg)	0.5–2.0	10–20	25	100–200
Tube Size	1.5 ml	1.5 ml	2.0 ml	15 ml
Genomic DNA Lysis Solution	100 µl	600 µl	750 µl	6 ml
RNase A Solution (4 mg/ml)	0.5 µl	3 µl	3.75 µl	30 µl
Protein Precipitation Solution	33 µl	200 µl	250 µl	2 ml
100% Isopropanol[1]	100 µl	600 µl	750 µl	6 ml
70% Ethanol	100 µl	600 µl	750 µl	6 ml
DNA Hydration Solution	10 µl	100 µl	150 µl	425 µl
Expected DNA Yield Range[2]	0.3–8 µg	5–8 µg	12–100 µg	50–800 µg
Average DNA Conc.[3]	415 µg/ml	400 µg/ml	400 µg/ml	1,000 µg/ml
Average DNA Yield	4.15 µg	40 µg	60 µg	425 µg

[1] If the DNA yield is expected to be low (for example, if the number of cells in the sample is low) add a DNA carrier such as glycogen to the isopropanol. Add 1 µl glycogen (20 mg/ml) per 600 µl 100% isopropanol.

[2] The expected DNA yield is based on average yields obtained from a variety of tissues. The yield of DNA may vary considerably depending on the tissue type.

[3] DNA concentration can be increased or decreased by adding more or less DNA Hydration Solution.

Whole Blood and Buffy Coat Reagent Volumes Scaling Table (0.05 ml to 10 ml Blood or 0.35 to 70 million white blood cells)

Blood Volume	50 µl	200 µl	500 µl	1 ml	5 ml	10 ml
Number White Cells[1]	$3.5x10^5$	$1.4x10^6$	$3.5 x10^6$	$7 x10^6$	$35 x10^6$	$70 x10^6$
Tube Size	1.5 ml	1.5 ml	1.5 ml	15 ml	50 ml	50 ml
RBC Lysis Solution	150 µl	600 µl	1.5 ml	3 ml	15 ml	30 ml
Genomic DNA Lysis Solution	50 µl	200 µl	500 µl	1 ml	5 ml	10 ml
RNase A Solution Optional	0.25 µl	1 µl	2.5 µl	5 µl	25 µl	50 µl
Protein Precipitation Solution	17 µl	67 µl	167 µl	333 µl	1.67 ml	3.33 ml
100% Isopropanol[2]	50 µl	200 µl	500 µl	1 ml	5 ml	10 ml
70% Ethanol	50 µl	200 µl	500 µl	1 ml	5 ml	10 ml
DNA Hydration Solution[3]	4 µl	17 µl	42 µl	83 µl	417 µl	833 µl
Theoretical DNA Yield (µg)[4]	1.7 µg	6.7 µg	16.8 µg	33.6 µg	168 µg	336 µg

[1] Cell number estimates assume an average of seven million white cells per ml of whole blood.

[2] If the DNA yield is expected to be low (for example, if the number of cells in the sample is low) add a DNA carrier such as glycogen to the isopropanol. Add 1 µl glycogen (20 mg/ml) per 600 µl isopropanol.

[3] DNA Hydration Solution volume gives a DNA concentration of approximately 400 µg/ml. Concentration can be increased by adding less DNA Hydration Solution or decreased by adding more DNA Hydration Solution.

[4] DNA yield assumes 6 pg DNA per human diploid cell and 80% recovery.

Section 6
Product Information

Catalog Number	Product Description
732-6340	**AquaPure Genomic DNA Isolation Kit**, (100), contains Genomic DNA Lysis Solution 35 ml, Protein Precipitation Solution 12 ml, DNA Hydration Solution 10 ml, RNase A Solution (4 mg/ml) 250 µl, manual
732-6345	**AquaPure Genomic DNA Blood Kit**, (100), contains RBC Lysis Solution 100 ml, Genomic DNA Lysis Solution 35 ml, Protein Precipitation Solution 12 ml, DNA Hydration Solution 10 ml, RNase A Solution (4 mg/ml) 250 µl, manual
732-6343	**AquaPure Genomic DNA Tissue Kit**, (100), contains Proteinase K (20 mg/ml) 175 µl, Genomic DNA Lysis Solution 35 ml, Protein Precipitation Solution 12 ml, DNA Hydration Solution 10 ml, RNase A Solution (4 mg/ml) 250 µl, manual
732-6370	**AquaPure RNA Isolation Kit**, (100), contains RNA Lysis Solution 35 ml, DNA/Protein Pelleting Solution 12 ml, RNA Hydration Solution 10 ml, manual
732-6371	**AquaPure RNA Blood Kit**, (100), contains RBC Lysis Solution 100 ml, RNA Lysis Solution 35 ml, DNA/Protein Pelleting Solution 12 ml, RNA Hydration Solution 10 ml, manual
732-6372	**RBC Lysis Buffer**, 100 ml
732-6348	**Proteinase K (20 mg/ml)**, 175 µl
732-6349	**RNase A Solution (4 mg/ml),** 250 µl

Bio-Rad Laboratories
2000 Alfred Nobel Dr., Hercules, CA 94547
510-741-1000

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