AquaPure RNA Isolation Kit

Catalog #

732-6370 RNA Isolation Kit
732-6371 RNA Blood Kit

BIO-RAD

Table of Contents

Section 1 Introduction 1

1.1 Overview 1
1.2 Quality Assurance 2
1.3 Kit Components 2
1.4 Materials Needed but Not Supplied..... 3
1.5 Storage and Stability 3

Section 2 Experimental Procedures..... 3

2.1 Recommendations for Best Results 3
2.2 Helpful Hints 4

Section 3 Protocols..... 5

3.1 Protocol for Extraction of RNA from Cultured Cells.. 6
3.2 Protocol for Extraction of RNA from Animal Tissues 7
3.3 Protocol for Extraction of RNA from Gram Negative Bacteria 8
3.4 Protocol for Extraction of RNA from Whole Blood or Bone Marrow..... 9
3.5 Protocol for Extraction of RNA from Plant Tissue.... 10
3.6 Protocol for Protein-DNA Precipitation, RNA Precipitation and RNA Hydration..... 10

Section 4 Troubleshooting 12

Section 5 Appendix.....16

Section 6 Product Information.....17

Section 1
Introduction

1.1 Overview

Purified RNA is required in RNA analysis procedures including Northern and dot blot hybridization, reverse transcription and amplification (RT-PCR), in vitro translation and ribonuclease protection assays. The AquaPure RNA Isolation Kit uses a proprietary modified salt precipitation procedure in combination with highly effective inhibitors of RNase activity to produce highly purified, intact RNA without the use of caustic or toxic reagents such as phenol or chloroform. The Quantum Prep AquaPure RNA Isolation kits provide rapid isolation of high quality purified RNA.

AquaPure RNA isolation kits provide purification of RNA from cells, bacteria and tissues. For RNA isolation from whole blood and bone marrow, the red blood cells are first lysed to facilitate their separation from the white blood cells. For RNA from isolated white blood cells, cultured and bacterial cells, animal and plant tissues, the cells are first lysed in the presence of a RNA preservative using an anionic detergent which solubilizes the cellular components. The RNA preservative works by limiting the activity of enzymes capable of digesting RNA (RNases) that are contained in the cell and elsewhere in the environment. Contaminating DNA and proteins are then removed by salt precipitation. Depending on the sample source, approximately 90–99.9% of the genomic DNA is removed using this procedure. Please consult the Appendix for information on treating samples with DNase when complete DNA removal is required. Total RNA is finally isolated by first precipitating with alcohol and then hydrated in RNase-free water.

1.2 Quality Assurance

All components of the AquaPure RNA isolation kits are subjected to a rigorous two-stage quality assurance testing process: "each kit component is tested to ensure the absence of RNases, RNA and microbial contamination. Next, the entire kit is tested for the ability to isolate RNA that meets our performance specifications for yield and quality."

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

1.3 Components

The AquaPure RNA isolation kit (catalog number 732-6370) contains materials and reagents sufficient for 100 preps.

35 ml	RNA Lysis Solution
12 ml	Protein/DNA Precipitation Solution
10 ml	RNA Hydration Solution

AquaPure blood RNA isolation kit (catalog number 732-6371) contains materials and reagents sufficient for 100 preps.

35 ml	RNA Lysis Solution
12 ml	Protein/DNA Precipitation Solution
10 ml	RNA Hydration Solution
100 ml	RBC Lysis Solution

1.4 Materials Required but Not Supplied

Reagents: 100% Isopropanol
70% ethanol
Liquid nitrogen (for tissues)
DEPC (diethyl pyrocarbonate) treated water

Equipment: Cell scrapers (for tissue culture cells)
Homogenizer (for tissues)
Mortar and pestle (for tissues)
Microfuge pestle (for tissues)
RNase-free microfuge tubes (2 per sample)

1.5 Storage and Stability

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

To avoid introducing RNases, great care must be taken in handling the reagents and purified RNA samples.

- If possible, work in an RNase-free environment; *e.g.*, use latex or vinyl gloves when handling reagents or RNA, keep reagent bottles closed if not in use and keep RNA samples on ice to prevent degradation by RNases.

- Use sterile plasticware and machine-packaged pipet tips. Pour tubes from an unopened bag (or bag marked For RNA Use Only) onto an RNase-free environment (such as plastic wrap).
- Surfaces can be made RNase-free by wiping down with a 0.5 M NaOH solution.
- Solutions (water and other solutions) not found in kit should be treated with 0.1% DEPC (diethyl pyrocarbonate). Add 0.1 ml DEPC for each 100 ml of the solution to be treated and shake vigorously to mix. Autoclave for 15 minutes at 15 lb/sq. in. on liquid cycle or incubate at least 12 hours at 37 °C and then heat to 100 °C for 15 minutes.
- It is a good practice to pulse spin tubes prior to opening the caps to reduce aerosols.
- Use aerosol resistant pipette tips when isolated RNA is to be used in amplification procedures.

Note: DEPC is suspected to be a carcinogen and should be handled with care. Always use gloves and open under a fume hood.

2.2 Helpful Hints

- This procedure is designed for isolation of total cell RNA from small samples of animal and plant tissue, gram-negative bacteria and blood. Table 1 has recommendations for starting amounts of material that may be used with this kit. Larger amounts may be used but it is important to proportionally increase the amount of RBC Lysis Solution, RNA Lysis Solution, and Protein/DNA Precipitation Solution.
- Use glycogen as an RNA carrier for samples with low cell numbers (fewer than 200,000 cells per 300 ml RNA Lysis Solution) or non-cellular body fluids. Add glycogen (1 ml 20

mg/ml glycogen per 600 ml isopropanol) to the isopropanol prior to or following the addition of the RNA-containing supernatant from the protein-DNA precipitation step.

- To scale up starting materials for RNA isolation, consult Appendix at back of manual.

TABLE 1. Recommended amounts of material for extraction of RNA

Material	RNA yield	Amount of sample
Mammalian cells (adherent)[1]	5–10 µg	1–2 x 10^6 cells
Animal tissue	0.5–10 µg	5–10 mg
Blood (normal)[2]	2–7 µg	300 µl
Plant tissue	0.5–3 µg	5–10 mg
Gram-negative bacteria[3]	10–30 µg	1 ml

[1] Depending on cell line, a confluent 60-mm plate of cells will typically contain 1–5 x 10^6 cells.

[2] Blood from a normal patient generally contains 5–10 x 10^6 white blood cells/ml. An equal volume of blood from a leukemic patient, which may contain up to 10 times this number of white blood cells, may also be processed.

[3] The number of cells in a one milliliter of culture will vary with species and strain; OD_{600} = 1 corresponds to about 8 x 10^8 cells/ml.

Section 3 Protocols

All steps are carried out at room temperature unless otherwise indicated. All centrifugations are at top speed (13,000–16,000 xg) in a microcentrifuge. Samples may be fresh or cell pellets may be stored frozen at -70° to -80 °C. All protocols are for specified sample sizes. For sample sizes larger than specified amounts see Appendix for scale up volumes.

Important Note: Protein-DNA Precipitation, RNA Precipitation and RNA Hydration steps are identical for all isolation protocols and found in Section 3.6.

3.1 Protocol for Extraction of RNA from Cultured Cells

Sample Collection

Collect cultured cells or other cells in suspension and place on ice. Determine the number of cells using a hemacytometer or other cell counter. Isolate RNA as quickly as possible.

RNA Isolation From 1–2 Million Cultured Cells Expected Yield: 5–10 µg RNA

Cell Lysis

1. Add 1-2 million cells suspended in balanced salt solution or culture medium to a 1.5 ml microfuge tube on ice.
2. Centrifuge at 13,000–16,000 x g for 5 seconds to pellet cells. Remove supernatant with a pipet leaving behind visible cell pellet and approximately 10–20 µl of residual liquid.
3. Vortex the tube to resuspend the cells in the residual supernatant (invert tube to check that the cell pellet has disappeared completely). This greatly facilitates cell lysis in Step 4.
4. Add 300 µl RNA Lysis Solution to the resuspended cells and pipet up and down no more than three times to lyse the cells. Alternatively, cultured cells that adhere to culture plates or flasks may be lysed directly on the plate by first removing culture media and then adding 300 µl RNA Lysis Solution. Swirl solution to cover entire plate and draw lysed cells up and down in a pipet three times before removing to a 1.5 ml microfuge tube.
5. Go to Section 3.6, Protein-DNA Precipitation, RNA Precipitation and RNA Hydration Protocol.

3.2 Protocol for Extraction of Animal Tissue

Sample Collection

Dissect tissue sample quickly. If freezing in liquid nitrogen, store at -70° to -80°C. If fresh tissue is being used, mince rapidly in cold PBS (phosphate buffered saline) and homogenize. Work very quickly and keep tissue on ice at all times, including when tissue is weighed.

RNA Isolation From 5–10 mg Fresh or Frozen Solid Tissue
Expected Yield: 0.5–10 µg RNA

Cell Lysis

1. Add 5–10 mg (0.005–0.010 g) fresh or frozen ground tissue to a 1.5 ml microfuge tube containing 300 µl RNA Lysis Solution. Grind frozen tissue finely in liquid nitrogen with a mortar and pestle. Keep tissue frozen until added to RNA Lysis Solution. Mince fresh tissue rapidly in cold PBS, and add 300 µl of RNA Lysis Solution.
2. Homogenize quickly using 5–10 strokes with a microfuge tube pestle.
3. Go to Section 3.6, Protein-DNA Precipitation, RNA Precipitation and RNA Hydration Protocol.

3.3 Extraction of RNA from Gram Negative Bacteria

Sample Collection

Samples may be either fresh or frozen as cell pellets at -70° to -80 °C. Collect overnight bacterial cultures and store on ice until use.

RNA Isolation From 0.5 ml Gram-Negative Bacterial Culture
Expected Yield: 10–30 µg RNA

Cell Lysis

1. Add 0.5 ml cell suspension (*e.g.*, overnight culture containing approximately 5 x 10^8 cells) to a 1.5 ml microfuge tube on ice.
2. Centrifuge at 13,000–16,000 x g in a microcentrifuge for 5 seconds to pellet cells and place tube back on ice. Remove as much supernatant as possible with a pipet.
3. Add 300 µl RNA Lysis Solution and gently pipet up and down 5 times to resuspend cells.
4. Incubate sample at 65 °C for 5 minutes to complete cell lysis. Cool sample to room temperature. Please note that incubation for 5 minutes at room temperature may be sufficient to lyse certain types of bacteria.
5. Go to Section 3.6, Protein-DNA Precipitation, RNA Precipitation and RNA Hydration Protocol.

3.4 Extraction of RNA from Whole Blood or Bone Marrow

Sample Collection

Collect whole blood and bone marrow in EDTA to prevent the blood from clotting. Process samples as soon as possible. For best results, store samples at 2–8 °C for not more than 24 hours prior to RNA isolation procedures.

RNA Isolation From 300 µl Whole Blood
Expected Yield: 2–7 µg RNA

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 once during the incubation.
2. Centrifuge for 20 seconds at 13,000–16,000 x g in a microcentrifuge. Remove supernatant with a pipet leaving behind the visible white cell pellet and 10–20 µl of residual liquid.
3. Vortex the tube vigorously to resuspend the white blood cells in the residual supernatant (invert tube to check that the white cell pellet has disappeared completely). This greatly facilitates cell lysis in Step 4 below.
4. Add 300 µl RNA Lysis Solution to the tube containing the resuspended cells and pipet up and down no more than three times to lyse the cells.
5. Go to Section 3.6, Protein-DNA Precipitation, RNA Precipitation and RNA Hydration Protocol.

3.5 Protocol for Extraction of RNA from Plant Tissue

Sample Collection

Collect fresh leaf or other tissue and keep on ice at all times, including when tissue is weighed. Isolate RNA as quickly as possible or store at -70 °C to -80 °C.

RNA Isolation from 5–10 mg Plant Tissue Expected Yield: 0.5–3 µg DNA

Cell Lysis

1. Add 5-10 mg (0.005–0.010 g) frozen ground tissue or fresh tissue to a 1.5 ml microfuge tube containing 300 µl RNA Lysis Solution. Grind frozen tissue finely in liquid nitrogen with a porcelain mortar and pestle. Keep tissue frozen until added to RNA Lysis Solution.
2. Homogenize quickly using 5–10 strokes with a microfuge tube pestle.
3. Go to Section 3.6, Protein-DNA Precipitation, RNA Precipitation and RNA Hydration Protocol.

3.6 Protocol for Protein-DNA Precipitation, RNA Precipitation and RNA Hydration

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

Protein-DNA Precipitation

1. Add 100 µl Protein-DNA Precipitation Solution to the cell lysate.

2. Invert tube gently 10 times and place tube into an ice bath for 5 minutes.
3. Centrifuge at 13,000–16,000 x g in a microcentrifuge for 3 minutes. The precipitated proteins and DNA will form a tight white pellet.

RNA Precipitation

1. Pour or pipet the supernatant containing the RNA (leaving behind the precipitated protein-DNA pellet) into a clean 1.5 ml microfuge tube containing 300 µl 100% isopropanol.
2. Mix the sample by inverting gently 25–30 times. Make sure sample is completely mixed.
3. Centrifuge at 13,000–16,000 x g in a microcentrifuge for 3 minutes; the RNA will be visible as a small, translucent pellet.
4. Pour off the supernatant and drain tube briefly on clean absorbent paper. Add 300 µl 70% ethanol and invert the tube several times to wash the RNA pellet.
5. Centrifuge at 13,000–16,000 x g in a microcentrifuge for 1 minute. Carefully pour off the ethanol.
6. Invert and drain the tube on clean absorbent paper and allow sample to air dry 10–15 minutes.

RNA Hydration

1. Add 50 µl RNA Hydration Solution.
2. Allow RNA to rehydrate for at least 30 minutes on ice. Alternatively, store RNA sample at -70° to -80°C until use.
3. Before use, vortex sample vigorously for 5 seconds and pulse spin. Pipet sample up and down several times to insure adequate mixing.
4. Store purified RNA sample at -70° to -80°C.

Section 4
Troubleshooting Guide

Possible Cause/Problem	Solutions or Suggestions
Red Blood Cell Lysis	
Incomplete lysis of red blood cells.	
Increased number of red blood cells.	Pellet cells and repeat red blood cell lysis step to lyse remaining red blood cells.
Incomplete cell lysis.	
Too many cells for the amount of RNA Lysis Solution used or presence of clumped cells.	In the situation where too many cells are present, add more RNA Lysis Solution and increase all other reagent volumes proportionately. (*Contact Technical Services if you need assistance in determining the correct reagent volumes.*) The presence of too many cells will inhibit cell lysis; the RNA Lysis Solution becomes very viscous and the cells clump. To prevent this problem, either count the cells with a hemacytometer or weigh tissue samples prior to starting the isolation procedure.
Incomplete lysis of cells isolated from a Ficoll gradient.	
Inadequate removal of Ficoll.	To remove Ficoll, wash cells three times with phosphate buffered saline prior to adding RNA Lysis Solution.
Protein-DNA Precipitation	
No protein-DNA pellet, soft or loose protein-DNA pellet.	
Centrifuge speed set too low.	Set centrifuge speed to the specified g force. For microfuge preps, set the speed to maximum. For other centrifuges, the speed will be set usually at 15,000 x g. If your centrifuge cannot attain a force of 15,000 x g, increase centrifugation time. *Please note that 15,000 x g and 15,000 rpm are* ***not*** *equivalent.*
	Extend centrifugation time until protein-DNA pellet is tight.

Possible Cause/Problem	Solutions or Suggestions
Sample or centrifuge rotor temperature too high.	Cool sample to room temperature (£ 21 °C) before adding Protein-DNA Precipitation Solution. After inverting to mix Protein-DNA Precipitation Solution with lysate, chill sample in an ice bath for at least 5 minutes and then centrifuge promptly. Reduce centrifugation temperature to 2–8 °C or chill rotor to 2–8 °C.
RNA Hydration	
Sample is slow to rehydrate.	
Sample was not mixed during the hydration step, the RNA pellet was overdried.	Mix samples periodically while hydrating samples on ice. Vortexing and pulse spinning the sample will aid in dissolving the RNA. Extend hydration time. RNA pellets that are overdried often require a longer rehydration time. Storing sample at -70 °C to -80 °C overnight or longer aids in hydration.
Poor RNA Quality	
Protein contamination in the rehydrated RNA sample.	
Exceeding recommended starting sample material.	Re-isolate RNA sample. To re-isolate the sample, first add 5 parts RNA Lysis Solution for every 1 part RNA Hydration Solution used to dissolve your sample. *Please note that it is very important that the sample be dissolved completely before proceeding*. Next add 2 parts Protein-DNA Precipitation Solution for every 1 part Hydration solution. Invert tube to mix and pellet proteins as specified. Precipitate the RNA with 6 parts isopropanol for every 1 part Hydration Solution. Wash precipitated RNA with 6 parts 70% ethanol and redissolve in Hydration solution. (*Contact Technical Service if you would like more detailed instructions or if you need assistance in determining the correct reagent volumes.*)

Possible Cause/Problem	Solutions or Suggestions
Excess contaminating protein in the purified RNA from whole blood.	
RBC Lysis Solution not removed adequately.	In the Cell Lysis step, remove as much RBC Lysis supernantant as possible using a pipet. Excess heme-containing supernatant may inhibit efficient protein removal in subsequent steps of the procedure.
Excess contaminating DNA in the purified RNA sample.	
Sample over handled during the Cell Lysis times step.	For cells, do not pipet more than 3 to mix the RNA Lysis Solution with the resuspended cells. For tissues, do not grind more than 5–10 times. Prolonged pipetting or grinding causes the DNA to shear. This reduces the DNA's ability to precipitate in the Protein-DNA precipitation step.
Isolated RNA is degraded.	
RNA degraded due to improper collection and storage of sample or purified sample is contaminated with RNase.	Collect and store samples using methods that preserve RNA integrity. It is recommended that samples be frozen in liquid nitrogen and then transferred to -70° to -80 °C for storage. To prevent RNase contamination during or following RNA purification, follow recommendations for working with RNA in Section 2.1, Recommendations for Best Results.
RNA degraded due to exceeding recommended starting sample material; this limits RNase inhibition by RNA isolation reagents.	Reduce starting material to recommended amounts by counting cells or weighing tissue.

Possible Cause/Problem	Solutions or Suggestions
Poor Yield	
Low RNA yield.	
Insufficient number of cells or cells with low RNA levels; or excessive number of cells in the starting sample or incomplete cell lysis.	Count cells or weigh tissue prior to beginning cell lysis step. It is very important to use the specified amount of starting sample material for a given volume of RNA isolation reagents. Too few cells may cause inefficient recovery of RNA during isopropanol precipitation while too many cells may overload the chemistry inhibiting complete cell lysis. In both situations the result is low yield. Grind samples in liquid nitrogen using a mortar and pestle to improve yields from tissues. Lower temperature and increase centrifugation time for samples with low cell numbers (fewer than 2×10^5 cells per 300 µl RNA Lysis Solution) to increase RNA recovery during the isopropanol precipitation step. After adding the RNA-containing supernatant from the Protein-DNA Precipitation step, incubate sample at -20 °C for 15–60 minutes (or overnight). Increase centrifugation time (to 15 minutes or more) and reduce centrifugation temperature to 2–8 °C during the isopropanol precipitation step.
Low RNA concentration.	
Lower than expected yield.	Reprecipitate RNA and rehydrate in a smaller volume of RNA Hydration solution. Reprecipitate RNA by adding 0.1 part Protein-DNA Precipitation Solution and two parts 95–100% ethanol for every 1 part RNA Hydration Solution used to hydrate your sample. Invert to mix and incubate on ice for 15 minutes. Centrifuge at 15,000 x g for 5 minutes, wash pellet with two parts 70% ethanol and air dry for 10–15 minutes. Rehydrate RNA sample in desired volume of RNA Hydration solution.

Section 5
Appendix

The following table gives guidelines for scaled-up RNA preps from various starting materials. Detailed protocols for these large scale preps are available from Bio-Rad Technical Service.

Scale Up Reagent Volumes

Sample Material	Sample Size	RNA Lysis Solution	Protein-DNA Solution	100 % Isopropanol	70% Ethanol	RNA Hydration Solution
Cultured Cells	1–2 x 10^6	300 µl	100 µl	300 µl	300 µl	50 µl
	10–20 x 10^6	3 ml	1 ml	3 ml	3 ml	200 µl
Animal Tissue	5–10 mg	300 µl	100 µl	300 µl	300 µl	50 µl
	50–100 mg	3 ml	1 ml	3 ml	3 ml	500 µl
Gram Negative Bacteria	500 µl	300 µl	100 µl	300 µl	300 µl	50 µl
	5 ml	3 ml	1 ml	3 ml	3 ml	500 µl
Plant Tissue	5–10 mg	300 µl	100 µl	300 µl	300 µl	50 µl
	50–100 mg	3 ml	1 ml	3 ml	3 ml	300 µl

Sample Material	Sample Size	RBC Lysis Solution	RNA Lysis Solution	Protein-DNA Solution	100% Isopropanol	70% Ethanol	RNA Hydration Solution
Whole Blood	300 µl	900 µl	300 µl	100 µl	300 µl	300 µl	50 µl
	3 ml	9 ml	3 ml	1 ml	3 ml	3 ml	100 µl

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

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4006179 Rev C