Genetic Tools & Reagents

Product Manual

Omni-Pure™ RNA Purification Systems

Blood & Bodily Fluids RNA Purification System

Tissue RNA Purification System

Gene Link™

Omni-Pure™ RNA Purification System

Version 4.X

Purification from blood, other bodily fluids, tissues and plants

Gene Link™

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Materials Supplied

Omni-Pure™ Blood & Bodily Fluids RNA Purification System

Product	Catalog No.	Size	Catalog No.	Size	Catalog No.	Size
Omni-Pure™ Blood & Bodily Fluids RNA Purification System	40-4081-05	50	40-4081-10	100	40-4081-50	500
	❑		❑		❑	
Materials Supplied						
GR1 Cell Suspension Solution	40-4082-05	50 ml	40-4082-01	100 ml	40-4082-50	500 ml
GR2 Cell Lysis Solution	40-4083-02	20 ml	40-4083-04	40 ml	40-4083-20	200 ml
GR3 DNA & Protein Precipitation Solution	40-4084-01	10 ml	40-4084-02	20 ml	40-4084-10	100 ml
GR4 RNA Reconstitution Solution	40-4085-01	10 ml	40-4085-01	10 ml	40-4085-05	50 ml

Omni-Pure™ Universal RNA Purification System

Product	Catalog No.	Size	Catalog No.	Size	Catalog No.	Size
Omni-Pure™ Universal RNA Purification System	40-4091-05	50	40-4091-10	100	40-4091-50	500
	❑		❑		❑	
Materials Supplied						
GR1 Cell Suspension Solution	40-4082-05	50 ml	40-4082-01	100 ml	40-4082-50	500 ml
GR2 Cell Lysis Solution	40-4083-02	20 ml	40-4083-04	40 ml	40-4083-20	200 ml
GR3 DNA & Protein Precipitation Solution	40-4084-01	10 ml	40-4084-02	20 ml	40-4084-10	100 ml
GR4 RNA Reconstitution Solution	40-4085-01	10 ml	40-4085-01	10 ml	40-4085-05	50 ml
*TCEP Solution; 0.5M	40-5116-01	100 µl	40-5116-02	200 µl	40-5116-10	1 ml
*DNase I ; 2 mg/ml	40-5111-02	200 µl	40-5111-10	1 ml	40-5111-10	2 x 1 ml
*Proteinase K; 10 mg/ml	40-5203-02	200 µl	40-5203-01	1 ml	40-5203-02	2 x 1 ml
*Glycogen; 10 mg/ml	40-5112-02	200 µl	40-5112-01	1 ml	40-5112-01	2 x 1 ml

***TCEP, DNase I, Proteinase K and Glycogen solutions should be stored frozen after use.**

Omni-Pure™ Blood & Bodily Fluids RNA Spin Column Purification System

Product	Catalog No.	Size	Catalog No.	Size	Catalog No.	Size
Omni-Pure™ Blood & Bodily Fluids RNA Spin Column Purification System	40-4080-05	50	40-4080-10	100	40-4080-50	500
	❑		❑		❑	
Materials Supplied						
GR1 Cell Suspension Solution	40-4082-05	50 ml	40-4082-01	100 ml	40-4082-50	500 ml
GR2 Cell Lysis Solution	40-4083-02	20 ml	40-4083-04	40 ml	40-4083-20	200 ml
GR3 DNA & Protein Precipitation Solution	40-4084-02	20 ml	40-4084-04	40 ml	40-4084-20	200 ml
GR4 RNA Reconstitution Solution	40-4085-01	10 ml	40-4085-01	10 ml	40-4085-02	20 ml
Spin Columns	40-4121-50	50	40-4121-01	100	40-4121-05	500

Omni-Pure™ Universal RNA Spin Column Purification System

Product	Catalog No.	Size	Catalog No.	Size	Catalog No.	Size
Omni-Pure™ Universal RNA Spin Column Purification System	40-4090-05	50	40-4090-10	100	40-4090-50	500
	❑		❑		❑	
Materials Supplied						
GR1 Cell Suspension Solution	40-4082-05	50 ml	40-4082-01	100 ml	40-4082-50	500 ml
GR2 Cell Lysis Solution	40-4083-02	20 ml	40-4083-04	40 ml	40-4083-20	200 ml
GR3 DNA & Protein Precipitation Solution	40-4084-02	20 ml	40-4084-04	40 ml	40-4084-20	200 ml
GR4 RNA Reconstitution Solution	40-4085-01	10 ml	40-4085-01	10 ml	40-4085-02	20 ml
*TCEP Solution; 0.5M	40-5116-01	100 µl	40-5116-02	200 µl	40-5116-10	1 ml
*DNase I ; 2 mg/ml	40-5111-02	200 µl	40-5111-10	1 ml	40-5111-10	2 x 1 ml
*Proteinase K; 10 mg/ml	40-5203-02	200 µl	40-5203-01	1 ml	40-5203-02	2 x 1 ml
*Glycogen; 10 mg/ml	40-5112-02	200 µl	40-5112-01	1 ml	40-5112-01	2 x 1 ml
Spin Columns	40-4121-50	50	40-4121-01	100	40-4121-05	500

***DNase I, Proteinase K and Glycogen solutions should be stored frozen after use.**

Product Description

Introduction

The Omni-Pure™ RNA Purification System provides an easy-to-use kit of optimized reagents and a rapid protocol to yield purified RNA. The RNA is suitable for all molecular biology applications and has been thoroughly tested. The Omni-Pure™ RNA purification system uses non-hazardous reagents and especially does not use the classic phenol-chloroform protocol. This kit has been tested for blood, animal tissues and plant tissue.

Sample Type

Blood is a good sample source to obtain ~1+ µg quantities of RNA from 300 µl sample volumes. This kit is particularly formulated to extract and purify RNA from 300 µl of blood and similar small sample sizes with all manipulations being carried out in 1.5 ml tubes. Multiple samples can be processed at the same time. Ultra pure RNA is obtained in less than 30 minutes from blood without DNase I treatment. The DNase treatment is optional and should be performed when an absolutely DNA free sample is required. Small amount of DNA is co-extracted with this procedure.

The Omni-Pure™ RNA Purification System can be used for RNA purification from all animal tissues, bodily fluid samples, plant tissues and pathogens. Slight modifications to the protocol can be instituted for the tissue types not listed in this manual based on the characteristics of the sample. For example, proteinase K and DNase treatments are not required for blood and other bodily fluid samples but should be used when required.

Protocol Principle

The basic principle used for the Omni-Pure™ RNA Purification System's first step is the preferential isolation of cells that contain RNA and disposal of the rest. In the case of blood, the white blood cells are separated and then lysed to release genomic DNA and RNA. DNA and protein are preferentially precipitated first followed by RNA precipitation. For tissues, the tissue is homogenized and the cells are lysed. The lysed cells contain proteins and other macromolecules in addition to genomic DNA. In the next step the proteins and DNA are precipitated leaving the RNA in the supernatant solution phase. The RNA is then precipitated with isopropanol. RNA purified by this procedure is adequately pure for reverse transcription based assays.

The single most common source of poor RNA yield is due to RNase contamination and the next reason is due to degraded sample. Please refer to the appendix for guidelines to avoid RNase contamination. An RNase free laboratory environment is a requirement.

Decontamination

All human and animal samples used for purification should be considered infectious. Proper decontamination protocols should be followed for eventual disposal. All waste materials should be properly decontaminated and disposed following institutional guidelines. A standard decontamination protocol is given in this manual for information only and is not a substitute for any other protocol established by the institution or OSHA. Household bleach is a readily available and effective disinfectant. Extended heating at 80^{o}C to 100^{o}C for 20 minutes or longer denatures and inactivates most pathogens.

Genotyping Method & Sample Requirements

Reverse transcription PCR based genotyping requires low quantities of RNA and 300 µl blood or 10-20 ml of buccal wash sample is sufficient to yield RNA for numerous amplification reactions. A few milliliters of amniotic fluid or a few milligrams of chorionic villi sample will suffice most prenatal diagnosis requirements.
RNA once purified should be reverse transcribed, amplified or stored frozen immediately to prevent degradation.

Omni-Pure™ RNA Purification System

IMPORTANT APPLICATION NOTES

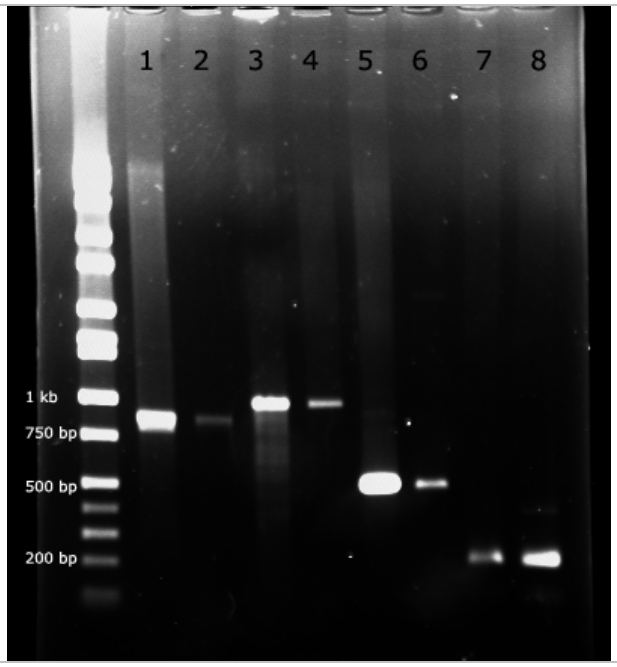
This kit version comprises of new optimized proprietary formulation. There are no hazardous components and the kit yields pure RNA from small amounts of sample size. It is particularly important to use only the recommended sample size and not to exceed it. Multiple purifications should be performed to process larger sample size. Using larger sample size will actually overload the system and not yield any purified product.
There is generally no need to add any RNase inhibitor, Proteinase or DNase. There is negligible amount of DNA co-extracted and this usually does not interfere with any down stream application. DNase digestion is recommended only when the RNA preparation has to be absolutely DNA free.

Summary

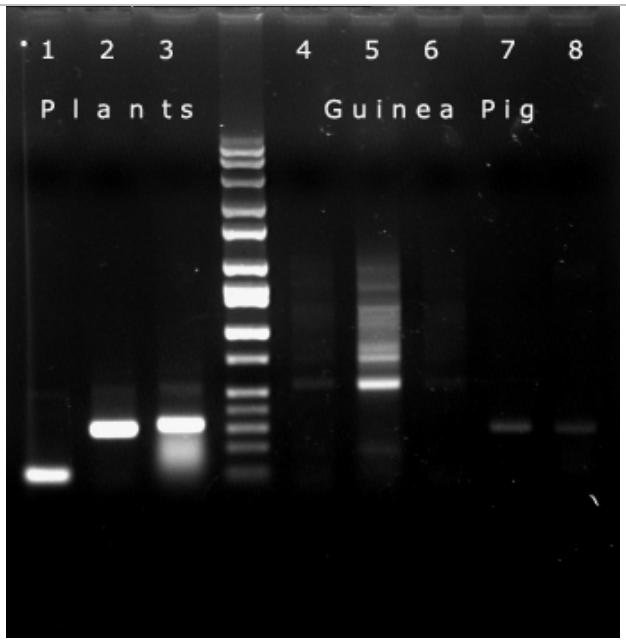
1. **Process small sample size and follow recommended sample size and volumes. Smaller sample size yields better RNA quality.**
2. **Perform multiple purifications to process larger sample size.**
3. **Some samples contain more protein and may require a second round of protein precipitation before proceeding to RNA precipitation.**
4. **Process purified RNA for reverse transcription immediately to prevent degradation.**
5. **Possible interruption steps if required are the Cell Lysis Step and the 70% ethanol steps. RNA is stable under these conditions for extended period of time.**

Typical Results

RNA was extracted from the samples indicated and processed for reverse transcription and amplification of fragments listed.



RT-PCR results from blood RNA: Lanes 1, 3, 5 & 7 are fluid phase RNA purification and lanes 2, 4, 6 and 8 are spin column RNA purification. Lane 1 & 2 beta actin 860 bp fragment; lanes 3 & 4 GAPDH 1009 bp near full length fragment; lanes 5 & 6 GAPDH 505 bp fragment and lanes 7 & 8 GAPDH 208 bp fragment.



RT-PCR results from Tomato leaf, potato and guinea pig tissues. Less than 1 mg sample size was processed for RNA extraction. Lane 1 Tomato leaf, Elongation Factor 1 (EF1) 101 bp; lane 2 Tomato leaf and lane 3 Potato Elongation factor 2 (EF2) 318. Lane 4 guinea pig heart, lane 5 liver and lane 6 lung GAPDH H 588 bp fragment; lane 7 heart and lane 8 liver beta actin A 289 bp fragment.

Omni-Pure™ RNA Purification System

Quick Protocol: Solution Phase & Spin Column Purification of RNA

from blood and other bodily fluids

Catalog No.: 40-4081-05; 40-4081-10; 40-4081-50; 40-4080-05; 40-4080-10; 40-4080-50

Sample volume example: 300 µl whole blood preferably in EDTA or citrate.

Please consult manual for details and background information. Sterile RNase free reagents, disposable pipet tips, tubes, and working environment required to obtain consistent RNA yield.

A. Sample & Reagent Preparation

1. Label two set of appropriate number of sterile RNase free 1.5 ml tubes. To one set add 900 µl ofGR1 Cell Suspension solution and 300 µl isopropanol (not provided) to the other set for RNA precipitation.

B. Cell Collection

1. Using a sterile RNase free filter tip pipet transfer 300 µl of blood sample to tubes containing 900 µl of GR1 Cell Suspension solution (Prepared in step A1 above). Mix thoroughly by gentle vortexing.
2. Incubate at room temperature for 10 minutes.
3. Centrifuge at 12K rpm for 30 seconds to pellet the cells.
4. Decant to remove the supernatant taking precaution not to dislodge the cell pellet.
5. Vortex the tube vigorously to completely resuspend the cells.

C. Cell Lysis

1. Add 300 µl GR2 Cell lysis solution to each tube and using the same pipet resuspend the cells by taking up and dispensing 3- 4 times.
2. Keep at room temperature for 3 minutes.

D. DNA & Protein Precipitation

1. Add 100 µl of GR3 DNA & Protein Precipitation solution. Mix by gentle inversion nearly 10 times. Chill tubes in ice bath for 5 minutes.
2. Centrifuge at 12K rpm for 5 minutes. A brown particulate pellet or DNA & protein should be visible.

⚠ Treat all bodily fluids, including blood and waste as hazardous material. Use appropriate safety procedures. Dispose following institutional guidelines. Refer to decontamination protocol in the manual.

⚠ Always use filter barrier pipette tips to prevent cross contamination.

ⓘ
- Prepare appropriately labeled tubes prior to starting procedure.
- It is convenient to add samples to tubes containing pre-aliquoted reagents.
- All samples should be at room temperature before processing.
- All centrifugation is carried out at room temperature.

RNA Precipitation

Fluid Phase

1. Transfer supernatant by decanting or by pipet to tubes containing 300 µl isopropanol. (Prepared in step A1 above).
2. Mix gently by inverting ~50 times. Centrifuge at 12K rpm for 5 minutes. RNA pellet is translucent and is usually visible.
3. Decant supernatant taking care not to dislodge the RNA pellet. Drain excess isopropanol by inverting on tissue paper.
4. Add 300 µl 70% ethanol. Mix gently and spin at 12K rpm for 3 minutes.
5. Decant supernatant taking care not to dislodge the RNA pellet. Drain excess ethanol by inverting on tissue paper.
6. Dry at room temperature for 10-15 minutes.

RNA Reconstitution

1. Add 25 µl GR4 RNA Reconstitution solution and leave in an ice bath for 20 minutes. Vortex gently and pulse spin.
2. Purified RNA should be reverse transcribed or amplified immediately, or stored at –20°C or preferably at –70°C.

Average yield of ~ 1 µg is obtained form 300 µl blood.

Spin Column

1. Transfer supernatant by decanting or by pipet to tubes containing 400 µl isopropanol. (Prepared in step A1 above).
2. Mix gently by inverting ~50 times. Transfer supernatant to appropriately labeled spin column with collection tube.
3. Centrifuge at 3K rpm for 5 minutes. Spin longer if you visibly notice any remaining liquid in spin column.

RNA Elution

1. Transfer spin column to new appropriately labeled RNA collection tube. Centrifuge empty spin column at 6K rpm for 3 minutes to dry the membrane.
2. Add 30 µl GR4 RNA Reconstitution solution to the middle of the spin column. Leave at room temperature for 3 minutes. Centrifuge at 12K rpm for 3 minutes.
3. Purified RNA should be reverse transcribed or amplified immediately, or stored at –20°C or preferably at –70°C.

Average yield of ~ 1 µg is obtained form 300 µl blood.

Omni-Pure™ RNA Purification System

Protocol: Purification of Genomic RNA from Blood & Bone Marrow

Sample: 300 µl blood or bone marrow **Average Yield: ~1 µg**

A. Initial Preparation

1. Label two sets of 1.5 ml tubes per sample.
2. Add 900 µl of GR1 solution (Cell Suspension Solution) to one tube for each sample.
3. Add 300 µl isopropanol (2-propanol) to one tube for each sample. Cap the tubes. This set will be used for RNA precipitation.

B. Cell Collection & Cell Lysis

1. To the tube containing 900 µl of GR1 solution (Cell Suspension Solution) transfer 300 µl of whole blood or bone marrow. Cap and gently mix by inversion. Incubate for 10 minutes at room temperature. Mix by inversion a few times during this incubation period. Incubate longer for fresh blood cells as they are intact and are not already lysed.
2. Centrifuge at 12K rpm for 20 seconds to pellet the white blood cells. A reddish white pellet should be clearly visible. Decant and discard supernatant leaving behind the last few droplets.
3. Completely resuspend the white blood cell pellet by vigorously vortexing the tube. Ensure that the pellet is completely resuspended.
4. To the resuspended cells add 300 µl of GR2 solution (Cell Lysis Solution). Mix by gentle vortexing. You will notice release of DNA by the thickening of the liquid in the sample.

C. DNA & Protein Precipitation

1. Add 100 µl of GR3 solution (DNA & Protein Precipitation Solution) to the sample in Cell Lysis Solution.
2. Mix by gentle inversion nearly 10 times. Chill tubes in ice bath for 5 minutes. Small particles of brown color will become visible at this stage.
3. Centrifuge at 12K rpm for 5 minutes. A clearly visible brown pellet containing DNA & proteins should be collected at the bottom of the tube.

⚠ Treat all bodily fluids and waste as hazardous material. Use appropriate safety procedures. Dispose following institutional guidelines. Refer to decontamination protocol in this manual.

ⓘ •Blood samples preferably should be un-coagulated and collected in EDTA (purple top) or ACD (yellow top) tubes.
•Use filter barrier pipette tips to prevent cross contamination.

ⓘ •White blood cell pellet should be completely resuspended.
•Add 1 µl of glycogen (10 mg/ml) as carrier to the isopropanol when the expected yield is below 1 µg.

•RNA can be reconstituted in smaller volume to achieve more concentrated solution.

RNA Precipitation

Fluid Phase	Spin Column
1. Transfer supernatant by decanting or by pipet to tubes containing 300 µl isopropanol. (Prepared in step A1 above). 2. Mix gently by inverting ~50 times. Centrifuge at 12K rpm for 5 minutes. RNA pellet is translucent and is usually visible. 3. Decant supernatant taking care not to dislodge the RNA pellet. Drain excess isopropanol by inverting on tissue paper. 4. Add 300 µl 70% ethanol. Mix gently and spin at 12K rpm for 3 minutes. 5. Decant supernatant taking care not to dislodge the RNA pellet. Drain excess ethanol by inverting on tissue paper. 6. Dry at room temperature for 10-15 minutes. **RNA Reconstitution** **1.** Add 25 µl GR4 RNA Reconstitution solution and leave in an ice bath for 20 minutes. Vortex gently and pulse spin. **2.** Purified RNA should be reverse transcribed or amplified immediately, or stored at –20°C or preferably at –70°C. **Average yield of ~ 1 µg is obtained form 300 µl blood.**	1. Transfer supernatant by decanting or by pipet to tubes containing 400 µl isopropanol. 2. Mix gently by inverting ~50 times. Transfer supernatant to appropriately labeled spin column with collection tube. 3. Centrifuge at 3K rpm for 5 minutes. Spin longer if you visibly notice any remaining liquid in spin column. **RNA Elution** 1. Transfer spin column to new appropriately labeled RNA collection tube. Centrifuge empty spin column at 6K rpm for 3 minutes to dry the membrane. 2. Add 30 µl GR4 RNA Reconstitution solution to the middle of the spin column. Leave at room temperature for 3 minutes. Centrifuge at 12K rpm for 3 minutes. 3. Purified RNA should be reverse transcribed or amplified immediately, or stored at –20°C or preferably at –70°C. **Average yield of ~ 1 µg is obtained form 300 µl blood.**

Omni-Pure™ RNA Purification System

Protocol: Purification of RNA from Amniotic Fluid

Sample: 1-6 ml Amniotic Fluid **Average Yield: 1 µg**

The number of exfoliated cells in amniotic fluid varies. This is dependent upon the subject and the timing of the fluid sampling. Standard amniotic fluid samples are generally taken within 15-18 weeks of gestation when the average cell number is about 200K cells per ml. Samples taken earlier will have drastically fewer cells. Culturing of the cells is recommended when it is not possible to obtain more samples. It is advised to spin down the fluid (cells) repeatedly in the same 1.5 ml tube to collect all the cells in one tube to be processed for RNA purification.

A. Sample Preparation & Cell Lysis

1. Spin 1.5 ml of amniotic fluid at 12K rpm for 20 seconds to pellet the cells. Decant supernatant. If you have more sample volume to process, add more to the same tube and collect cells by repeated spinning.
2. After the last spin, decant and discard supernatant leaving behind the last few droplets. Do not totally remove the supernatant.
3. Completely resuspend the cell pellet by vigorously vortexing the tube. Ensure that the pellet is completely resuspended.
4. To the resuspended cells add 300 µl of GR2 solution (Cell Lysis Solution). Mix by gentle vortexing. You will notice release of DNA by the thickening of the liquid in the sample.

B. DNA & Protein Precipitation

1. Add 100 µl of GR3 solution (DNA & Protein Precipitation Solution) to the sample in Cell Lysis Solution.
2. Mix by gentle inversion nearly 10 times. Chill tubes in ice bath for 5 minutes. Small particles of brown color will become visible at this stage.
3. Centrifuge at 12K rpm for 5 minutes to pellet the precipitated DNA & proteins. A clearly visible brown pellet containing DNA & proteins should be collected at the bottom of the tube.

•Use filter barrier pipette tips to prevent cross contamination.

Treat all bodily fluids and waste as hazardous material. Use appropriate safety procedures. Dispose following institutional guidelines. Refer to decontamination protocol in this manual.

•Cell pellet should be completely resuspended in Cell Lysis Solution.
•Samples may be stored at this stage for processing later.

•Small brown particles should be visible as an indication of DNA & protein being precipitated.
• All samples should be at room temperature before processing.

RNA Precipitation

Fluid Phase

1. Transfer supernatant by decanting or by pipet to tubes containing 300 µl isopropanol. (Prepared in step A1 above).
2. Mix gently by inverting ~50 times. Centrifuge at 12K rpm for 5 minutes. RNA pellet is translucent and is usually visible.
3. Decant supernatant taking care not to dislodge the RNA pellet. Drain excess isopropanol by inverting on tissue paper.
4. Add 300 µl 70% ethanol. Mix gently and spin at 12K rpm for 3 minutes.
5. Decant supernatant taking care not to dislodge the RNA pellet. Drain excess ethanol by inverting on tissue paper.
6. Dry at room temperature for 10-15 minutes.

RNA Reconstitution

1. Add 25 µl GR4 RNA Reconstitution solution and leave in an ice bath for 20 minutes. Vortex gently and pulse spin.
2. Purified RNA should be reverse transcribed or amplified immediately, or stored at –20°C or preferably at –70°C.

Average yield of ~ 1 µg is obtained form 300 µl blood.

Spin Column

1. Transfer supernatant by decanting or by pipet to tubes containing 400 µl isopropanol.
2. Mix gently by inverting ~50 times. Transfer supernatant to appropriately labeled spin column with collection tube.
3. Centrifuge at 3K rpm for 5 minutes. Spin longer if you visibly notice any remaining liquid in spin column.

RNA Elution

1. Transfer spin column to new appropriately labeled RNA collection tube. Centrifuge empty spin column at 6K rpm for 3 minutes to dry the membrane.
2. Add 30 µl GR4 RNA Reconstitution solution to the middle of the spin column. Leave at room temperature for 3 minutes. Centrifuge at 12K rpm for 3 minutes.
3. Purified RNA should be reverse transcribed or amplified immediately, or stored at –20°C or preferably at –70°C.

Average yield of ~ 1 µg is obtained form 300 µl blood.

Omni-Pure™ RNA Purification System

Protocol: Purification of DNA from Chorionic Villi

Sample: 5-10 mg Chorionic Villi **Average Yield: 1-2 µg**

A. Sample Preparation & Cell Lysis

1. Sample Washing: Transfer chorionic villi sample to a 1.5 ml tube and add 400 µl GR1. Spin at 12K rpm for 20 seconds to pellet the sample. Decant supernatant. Repeat this step again.
2. Add 300 µl of GR2 solution (Cell Lysis Solution), vortex vigorously to break up the tissue. If required, crush the remaining tissue using a sterile pipet tip.
3. Incubate at 65°C for 10 minutes to facilitate dissolution and complete lysis.
4. *Optional Step:* Perform this step if you observe that the cells are not completely lysed. You will notice release of DNA by the thickening of the liquid in the sample upon complete cell lysis. Add 4 µl of Proteinase K (10mg/ml) and incubate at 55°C for 1 hour to complete lysis.

B. DNA & Protein Precipitation

1. Add 100 µl of GR3 solution (DNA & Protein Precipitation Solution) to the sample in Cell Lysis Solution.
2. Mix by gentle inversion nearly 10 times. Chill tubes in ice bath for 5 minutes. Particles of brown color will become visible at this stage.
3. Centrifuge at 12K rpm for 5 minutes to pellet the precipitated DNA & proteins. A clearly visible brown pellet containing DNA & proteins should be collected at the bottom of the tube.

•Use filter barrier pipette tips to prevent cross contamination.

Treat all bodily fluids and waste as hazardous material. Use appropriate safety procedures. Dispose following institutional guidelines. Refer to decontamination protocol in this manual.

•Cell pellet should be completely resuspended in Cell Lysis Solution.

•Samples may be stored at this stage for processing later.

RNA Precipitation

Fluid Phase	Spin Column
1. Transfer supernatant by decanting or by pipet to tubes containing 300 µl isopropanol. (Prepared in step A1 above). 2. Mix gently by inverting ~50 times. Centrifuge at 12K rpm for 5 minutes. RNA pellet is translucent and is usually visible. 3. Decant supernatant taking care not to dislodge the RNA pellet. Drain excess isopropanol by inverting on tissue paper. 4. Add 300 µl 70% ethanol. Mix gently and spin at 12K rpm for 3 minutes. 5. Decant supernatant taking care not to dislodge the RNA pellet. Drain excess ethanol by inverting on tissue paper. 6. Dry at room temperature for 10-15 minutes. **RNA Reconstitution** **1.** Add 25 µl GR4 RNA Reconstitution solution and leave in an ice bath for 20 minutes. Vortex gently and pulse spin. **2.** Purified RNA should be reverse transcribed or amplified immediately, or stored at –20°C or preferably at –70°C. **Average yield of ~ 1 to 2 µg is obtained.**	1. Transfer supernatant by decanting or by pipet to tubes containing 400 µl isopropanol. 2. Mix gently by inverting ~50 times. Transfer supernatant to appropriately labeled spin column with collection tube. 3. Centrifuge at 3K rpm for 5 minutes. Spin longer if you visibly notice any remaining liquid in spin column. **RNA Elution** 1. Transfer spin column to new appropriately labeled RNA collection tube. Centrifuge empty spin column at 6K rpm for 3 minutes to dry the membrane. 2. Add 30 µl GR4 RNA Reconstitution solution to the middle of the spin column. Leave at room temperature for 3 minutes. Centrifuge at 12K rpm for 3 minutes. 3. Purified RNA should be reverse transcribed or amplified immediately, or stored at –20°C or preferably at –70°C. **Average yield of ~ 1 to 2 µg is obtained.**

Omni-Pure™ RNA Purification System

Protocol: Purification of RNA from other Bodily Fluids

Sample: 100 µl CSF, Plasma, Saliva, Milk, Semen, etc. **Average Yield: ~1 µg**

A. Sample Preparation & Cell Lysis

1. Plan on the number of samples to be processed and the volume of samples. Consider that a maximum of 100 µl of sample can be accommodated in each 1.5 ml tube. Label two (duplicate) sets of tubes. Add 500 µl of GR2 (Cell Lysis Solution) to set 1 and 600 µl 100% isopropanol (2-propanol) to set 2.
2. Transfer 100 µl bodily fluid samples to each 1.5 ml tube of set 1 containing 500 µl of GR2 (Cell Lysis Solution). Vortex vigorously to break up the cells.
3. Incubate at 65°C for 10 minutes to facilitate dissolution and complete lysis.
4. *Optional Step:* Perform this step if you observe that the cells are not completely lysed. You will notice release of DNA by the thickening of the liquid in the sample upon complete cell lysis. Add 4 µl of Proteinase K (10mg/ml) and incubate at 55°C for 1 hour to complete lysis.

B. DNA & Protein Precipitation

1. Add 100 µl of GR3 solution (DNA & Protein Precipitation Solution) to the sample in Cell Lysis Solution.
2. Mix by gentle inversion nearly 10 times. Chill tubes in ice bath for 5 minutes. Small particles of brown color will become visible at this stage.
3. Centrifuge at 12K rpm for 5 minutes to pellet the precipitated DNA & proteins. A clearly visible brown pellet containing DNA & proteins should be collected at the bottom of the tube.

•Use filter barrier pipette tips to prevent cross contamination.

Treat all bodily fluids and waste as hazardous material. Use appropriate safety procedures. Dispose following institutional guidelines. Refer to decontamination protocol in this manual.

•Cell pellet should be completely resuspended in Cell Lysis Solution.
•Samples may be stored at this stage for processing later.

•Add 1 µl of glycogen (10 mg/ml) as carrier to the isopropanol when the expected yield is below 1 µg.
•RNA can be reconstituted in smaller volume to achieve more concentrated solution.

RNA Precipitation

Fluid Phase	Spin Column
1. Transfer supernatant by decanting or by pipet to tubes containing 300 µl isopropanol. (Prepared in step A1 above). 2. Mix gently by inverting ~50 times. Centrifuge at 12K rpm for 5 minutes. RNA pellet is translucent and is usually visible. 3. Decant supernatant taking care not to dislodge the RNA pellet. Drain excess isopropanol by inverting on tissue paper. 4. Add 300 µl 70% ethanol. Mix gently and spin at 12K rpm for 3 minutes. 5. Decant supernatant taking care not to dislodge the RNA pellet. Drain excess ethanol by inverting on tissue paper. 6. Dry at room temperature for 10-15 minutes. **RNA Reconstitution** **1.** Add 25 µl GR4 RNA Reconstitution solution and leave in an ice bath for 20 minutes. Vortex gently and pulse spin. **2.** Purified RNA should be reverse transcribed or amplified immediately, or stored at –20°C or preferably at –70°C. **Average yield of ~ 1 µg is obtained.**	1. Transfer supernatant by decanting or by pipet to tubes containing 400 µl isopropanol. 2. Mix gently by inverting ~50 times. Transfer supernatant to appropriately labeled spin column with collection tube. 3. Centrifuge at 3K rpm for 5 minutes. Spin longer if you visibly notice any remaining liquid in spin column. **RNA Elution** 1. Transfer spin column to new appropriately labeled RNA collection tube. Centrifuge empty spin column at 6K rpm for 3 minutes to dry the membrane. 2. Add 30 µl GR4 RNA Reconstitution solution to the middle of the spin column. Leave at room temperature for 3 minutes. Centrifuge at 12K rpm for 3 minutes. 3. Purified RNA should be reverse transcribed or amplified immediately, or stored at –20°C or preferably at –70°C. **Average yield of ~ 1 µg is obtained.**

Omni-Pure™ RNA Purification System

Protocol: Purification of RNA from Buccal Wash

Sample: 10-20 ml Buccal Wash | **Average Yield: 1 µg**

A. Sample Preparation & Cell Lysis

1. Buccal wash samples can be conveniently collected using commercial mouth wash solutions. Alternatively, clean water can be used to obtain a buccal wash sample. It is important to rotate the wash solution in the mouth to get the maximum number of cells. Spin the buccal wash sample in a 50 ml tube at 5K rpm for 5 minutes to pellet the cells. Decant supernatant.
2. Resuspend the cells in 900 µl of GR1 (Cell Suspension solution) and transfer to a 1.5 ml tube. Spin at 12K rpm for 2 minutes to pellet the cells. Decant and discard supernatant leaving behind the last few droplets. Do not totally remove the supernatant.
3. Completely resuspend the cell pellet by vigorously vortexing the tube. Ensure that the pellet is completely resuspended.

B. DNA & Protein Precipitation

1. Add 100 µl of GR3 solution (DNA & Protein Precipitation Solution) to the sample in Cell Lysis Solution.
2. Mix by gentle inversion nearly 10 times. Chill tubes in ice bath for 5 minutes. Small particles of brown color will become visible at this stage.
3. Centrifuge at 12K rpm for 5 minutes to pellet the precipitated DNA & proteins. A clearly visible brown pellet containing DNA & proteins should be collected at the bottom of the tube.

•Use filter barrier pipette tips to prevent cross contamination.

Treat all bodily fluids and waste as hazardous material. Use appropriate safety procedures. Dispose following institutional guidelines. Refer to decontamination protocol in this manual.

•Cell pellet should be completely resuspended in Cell Lysis Solution.
•Samples may be stored at this stage for processing later.

• All samples should be at room temperature before processing.

RNA Precipitation

Fluid Phase

1. Transfer supernatant by decanting or by pipet to tubes containing 300 µl isopropanol. (Prepared in step A1 above).
2. Mix gently by inverting ~50 times. Centrifuge at 12K rpm for 5 minutes. RNA pellet is translucent and is usually visible.
3. Decant supernatant taking care not to dislodge the RNA pellet. Drain excess isopropanol by inverting on tissue paper.
4. Add 300 µl 70% ethanol. Mix gently and spin at 12K rpm for 3 minutes.
5. Decant supernatant taking care not to dislodge the RNA pellet. Drain excess ethanol by inverting on tissue paper.
6. Dry at room temperature for 10-15 minutes.

RNA Reconstitution

1. Add 25 µl GR4 RNA Reconstitution solution and leave in an ice bath for 20 minutes. Vortex gently and pulse spin.
2. Purified RNA should be reverse transcribed or amplified immediately, or stored at –20°C or preferably at –70°C.

Average yield of ~ 1 µg is obtained.

Spin Column

1. Transfer supernatant by decanting or by pipet to tubes containing 400 µl isopropanol.
2. Mix gently by inverting ~50 times. Transfer supernatant to appropriately labeled spin column with collection tube.
3. Centrifuge at 3K rpm for 5 minutes. Spin longer if you visibly notice any remaining liquid in spin column.

RNA Elution

1. Transfer spin column to new appropriately labeled RNA collection tube. Centrifuge empty spin column at 6K rpm for 3 minutes to dry the membrane.
2. Add 30 µl GR4 RNA Reconstitution solution to the middle of the spin column. Leave at room temperature for 3 minutes. Centrifuge at 12K rpm for 3 minutes.
3. Purified RNA should be reverse transcribed or amplified immediately, or stored at –20°C or preferably at –70°C.

Average yield of ~ 1 µg is obtained.

Omni-Pure™ RNA Purification System

Protocol: Purification of RNA from Cultured Cells

Sample: Cultured Cells (maximum 2 x 10^6 cells) **Average Yield: 1-2 µg**

A. Harvest cells

1. Cells grown in Suspension
   Spin down the appropriate number of cells (maximum 2 x 10^6 cells) for 5 minutes at 3K rpm in a 1.5 ml tube. Decant and discard the supernatant, taking care not to dislodge the cell pellet.. Go to step B.
2. Cells grown in a Monolayer
   Cells grown in a monolayer can be detached from the culture flask by either trypsinization or using a cell scraper.
   Harvest cells and transfer cells (maximum 2 x 10^6 cells) to a 1.5 ml tube and centrifuge for 5 minutes at 300x g. Decant and discard the supernatant.

B. Cell Lysis

1. Resuspend the cells in residual supernatant by vortexing. Resuspension facilitates the subsequent lysis step by minimizing the formation of cell clumps.
2. Add 600 µl of solution GR2 (Cell Lysis Solution). Mix by pipeting up and down several times. You will notice release of DNA by the thickening of the liquid in the sample.
   Optional Step: Perform this step if you observe that the cells are not completely lysed. You will notice release of DNA by the thickening of the liquid in the sample upon complete cell lysis. Add 4 µl of Proteinase K (10mg/ml) and incubate at 55°C for 1 hour to complete lysis.

C. DNA & Protein Precipitation

1. Add 200 µl of GR3 solution (DNA & Protein Precipitation Solution) to the sample in Cell Lysis Solution.
2. Vortex vigorously for 20 seconds. Small particles of pale yellow color will become visible at this stage.
3. Centrifuge at 12K rpm for 5 minutes to pellet the precipitated proteins. A clearly visible pale yellow pellet containing proteins should be collected at the bottom of the tube.

•Use filter barrier pipette tips to prevent cross contamination.

⚠ Treat all bodily fluids and waste as hazardous material. Use appropriate safety procedures. Dispose following institutional guidelines. Refer to decontamination protocol in this manual.

•Cell pellet should be completely resuspended in Cell Lysis Solution.
•Samples may be stored at this stage for processing later.

•Cooling the sample tubes containing the DNA & protein precipitation solution on ice facilitates the formation of a tight pellet.

•Add 1 µl of glycogen (10 mg/ml) as carrier to the isopropanol when the expected yield of RNA is below 1 µg.

RNA Precipitation

Fluid Phase

1. Transfer supernatant by decanting or by pipet to tubes containing 300 µl isopropanol. (Prepared in step A1 above).
2. Mix gently by inverting ~50 times. Centrifuge at 12K rpm for 5 minutes. RNA pellet is translucent and is usually visible.
3. Decant supernatant taking care not to dislodge the RNA pellet. Drain excess isopropanol by inverting on tissue paper.
4. Add 300 µl 70% ethanol. Mix gently and spin at 12K rpm for 3 minutes.
5. Decant supernatant taking care not to dislodge the RNA pellet. Drain excess ethanol by inverting on tissue paper.
6. Dry at room temperature for 10-15 minutes.

RNA Reconstitution

1. Add 25 µl GR4 RNA Reconstitution solution and leave in an ice bath for 20 minutes. Vortex gently and pulse spin.
2. Purified RNA should be reverse transcribed or amplified immediately, or stored at –20°C or preferably at –70°C.

Average yield of ~ 1 to 3 µg is obtained.

Spin Column

1. Transfer supernatant by decanting or by pipet to tubes containing 400 µl isopropanol.
2. Mix gently by inverting ~50 times. Transfer supernatant to appropriately labeled spin column with collection tube.
3. Centrifuge at 3K rpm for 5 minutes. Spin longer if you visibly notice any remaining liquid in spin column.

RNA Elution

1. Transfer spin column to new appropriately labeled RNA collection tube. Centrifuge empty spin column at 6K rpm for 3 minutes to dry the membrane.
2. Add 30 µl GR4 RNA Reconstitution solution to the middle of the spin column. Leave at room temperature for 3 minutes. Centrifuge at 12K rpm for 3 minutes.
3. Purified RNA should be reverse transcribed or amplified immediately, or stored at –20°C or preferably at –70°C.

Average yield of ~ 1 to 3 µg is obtained.

Omni-Pure™ RNA Purification System

Protocol: Purification of RNA from Tissue

Sample: Animal Tissue (5-10 mg) **Average Yield: 1-2 µg**

A. Tissue Lysis

1. Place 5-10 mg fresh or frozen animal tissue into a 1.5 ml tube containing 600 µl of GR2 (Cell Lysis Solution)**.** If possible, fresh tissue should be finely minced and frozen tissue crushed (preferably in liquid nitrogen) with a mortar and pestle to facilitate the lysis procedure.
2. To the lysate, add 4 µl Proteinase K Solution (10 mg/ml) and mix by gentle inversion 25-30 times. Incubate at 55°C for 60 minutes or until tissue has dissolved. If possible, the tube should be mixed periodically by inversion during the incubation. Some undigested material may be present at the end of the incubation, which will be removed during the protein precipitation step.

B. DNA & Protein Precipitation

1. Add 200 µl of GR3 solution (DNA & Protein Precipitation Solution) to the sample in Cell Lysis Solution.
2. Vortex vigorously for 20 seconds. Small particles of pale yellow color will become visible at this stage.
3. Centrifuge at 12K rpm for 5 minutes to pellet the precipitated proteins. A clearly visible pale yellow pellet containing proteins should be collected at the bottom of the tube.

•Use filter barrier pipette tips to prevent cross contamination.

⚠ •All bodily fluids and tissue samples are to be considered infectious and hazardous.

• All waste materials should be properly decontaminated and disposed following institutional guidelines.

•Cell pellet should be completely resuspended in Cell Lysis Solution.

•Samples may be stored at this stage for processing later.

•Vigorous vortexing is required at the protein precipitation step.

•Cooling the sample tubes containing the DNA & protein precipitation solution on ice facilitates the formation of a tight pellet.

RNA Precipitation

Fluid Phase	Spin Column
1. Transfer supernatant by decanting or by pipet to tubes containing 300 µl isopropanol. (Prepared in step A1 above). 2. Mix gently by inverting ~50 times. Centrifuge at 12K rpm for 5 minutes. RNA pellet is translucent and is usually visible. 3. Decant supernatant taking care not to dislodge the RNA pellet. Drain excess isopropanol by inverting on tissue paper. 4. Add 300 µl 70% ethanol. Mix gently and spin at 12K rpm for 3 minutes. 5. Decant supernatant taking care not to dislodge the RNA pellet. Drain excess ethanol by inverting on tissue paper. 6. Dry at room temperature for 10-15 minutes. **RNA Reconstitution** **1.** Add 25 µl GR4 RNA Reconstitution solution and leave in an ice bath for 20 minutes. Vortex gently and pulse spin. **2.** Purified RNA should be reverse transcribed or amplified immediately, or stored at –20°C or preferably at –70°C. **Average yield of ~ 1 to 3 µg is obtained.**	1. Transfer supernatant by decanting or by pipet to tubes containing 400 µl isopropanol. 2. Mix gently by inverting ~50 times. Transfer supernatant to appropriately labeled spin column with collection tube. 3. Centrifuge at 3K rpm for 5 minutes. Spin longer if you visibly notice any remaining liquid in spin column. **RNA Elution** 1. Transfer spin column to new appropriately labeled RNA collection tube. Centrifuge empty spin column at 6K rpm for 3 minutes to dry the membrane. 2. Add 30 µl GR4 RNA Reconstitution solution to the middle of the spin column. Leave at room temperature for 3 minutes. Centrifuge at 12K rpm for 3 minutes. 3. Purified RNA should be reverse transcribed or amplified immediately, or stored at –20°C or preferably at –70°C. **Average yield of ~ 1 to 3 µg is obtained.**

Omni-Pure™ RNA Purification System

Protocol: Purification of RNA from Plant Tissue

Sample: Plant Tissue (5-10 mg) **Average Yield: 1-5 µg**

A. Tissue Lysis

Samples may be fresh, frozen or dried. Fresh tissue can be stored for up to 5 days at 4°C. Frozen tissue should be stored at -70°C. Dried tissue can be stored at room temperature. Fresh or frozen tissue should be finely crushed with a mortar and pestle in liquid nitrogen prior to RNA purification. If liquid nitrogen is not available, then the tissue can be homogenized during the cell lysis step using 30-50 strokes with a 1.5 ml tube pestle. Dried tissue can be ground with a mortar and pestle at room temperature. From 10-30 mg of fresh/frozen tissue or 5-10 mg of dried tissue the expected yield of RNA is 2-15 µg.

1. Add the tissue to a 1.5 ml tube.
2. Add 600 µl of GR2 (Cell Lysis Solution) to the sample. When using dried tissue, vortex for a few seconds to give the tissue a chance to rehydrate.
3. Incubate the tissue lysate at 65°C for 60 minutes. At 15 minute intervals, mix the contents of the tube by inversion 10 times.

B. DNA & Protein Precipitation

1. Add 200 µl of GR3 solution (DNA & Protein Precipitation Solution) to the sample in Cell Lysis Solution.
2. Vortex vigorously for 20 seconds. Small particles of pale yellow color will become visible at this stage.
3. Centrifuge at 12K rpm for 5 minutes to pellet the precipitated proteins. A clearly visible pale yellow pellet containing proteins should be collected at the bottom of the tube.

•Use filter barrier pipette tips to prevent cross contamination.
•Cell pellet should be completely resuspended in Cell Lysis Solution.
•Samples may be stored at this stage for processing later.

•Vigorous vortexing is required at the protein precipitation step.
•Small pale yellow particles should be visible as an indication of protein being precipitated.

•Cooling the sample tubes containing the DNA & protein precipitation solution on ice facilitates the formation of a tight pellet.

•RNA can be reconstituted in smaller volume to achieve more concentrated solution.

RNA Precipitation

Fluid Phase	Spin Column
1. Transfer supernatant by decanting or by pipet to tubes containing 300 µl isopropanol. (Prepared in step A1 above). 2. Mix gently by inverting ~50 times. Centrifuge at 12K rpm for 5 minutes. RNA pellet is translucent and is usually visible. 3. Decant supernatant taking care not to dislodge the RNA pellet. Drain excess isopropanol by inverting on tissue paper. 4. Add 300 µl 70% ethanol. Mix gently and spin at 12K rpm for 3 minutes. 5. Decant supernatant taking care not to dislodge the RNA pellet. Drain excess ethanol by inverting on tissue paper. 6. Dry at room temperature for 10-15 minutes. **RNA Reconstitution** **1.** Add 25 µl GR4 RNA Reconstitution solution and leave in an ice bath for 20 minutes. Vortex gently and pulse spin. **2.** Purified RNA should be reverse transcribed or amplified immediately, or stored at –20°C or preferably at –70°C. **Average yield of ~ 1 to 3 µg is obtained.**	1. Transfer supernatant by decanting or by pipet to tubes containing 400 µl isopropanol. 2. Mix gently by inverting ~50 times. Transfer supernatant to appropriately labeled spin column with collection tube. 3. Centrifuge at 3K rpm for 5 minutes. Spin longer if you visibly notice any remaining liquid in spin column. **RNA Elution** 1. Transfer spin column to new appropriately labeled RNA collection tube. Centrifuge empty spin column at 6K rpm for 3 minutes to dry the membrane. 2. Add 30 µl GR4 RNA Reconstitution solution to the middle of the spin column. Leave at room temperature for 3 minutes. Centrifuge at 12K rpm for 3 minutes. 3. Purified RNA should be reverse transcribed or amplified immediately, or stored at –20°C or preferably at –70°C. **Average yield of ~ 1 to 3 µg is obtained.**

Troubleshooting

Problem	Protocol Step	Reasons and Suggestions
Brown or dark colored pellet	DNA & Protein precipitation	Incomplete protein precipitation. Repeat protein precipitation step. Too much sample or protein in sample.
Low RNA yield	Sample preparation	RNA in sample was degraded prior to RNA purification. 1. Too much sample volume used. The reagent volume per sample purification is optimized based on the specified sample size and volume. Larger sample size will not yield more as the capacity will be limited. Multiple samples should be processed to process larger volume. 2. Sample was not adequately protected from RNA degradation. RNase free environment, disposable plastic wares, equipment and reagents are an absolute requirement for obtaining good RNA yield. 3. Ensure that sample was transported rapidly and collected in RNase free container. 4. Ensure that sample was stored properly; preferably frozen if the sample is not processed within 24 hrs.
Low RNA yield	Cell lysis	RNA in sample was not lysed completely. 1. Ensure that correct amount of solution GR2 was added. 2. The sample was not completely lysed. Presence of too many cells release DNA and proteins that compete with RNA extraction. 3. Too large sample used. A larger than recommended sample may actually yield lower RNA. Multiple purifications are advised to process larger sample volume.
Low RNA yield	RNA precipitation	RNA in sample was not completely recovered after isopropanol precipitation. 1. Recovery of low amount of RNA is assisted by adding glycogen as a carrier. 2. The RNA was incompletely precipitated. The volume of isopropanol should not be more than the volume of aqueous sample. Equal volume of isopropanol should be added.
Low or no RNA yield		RNA degradation. 1. The single most common reason for problems with RNA purification is not adequately performing the purification in an RNase free manner. Follow the protocol given in the appendix for 'Avoiding RNase Contamination."

Appendix

Protocols for Avoiding Ribonuclease Contamination

Ribonuclease present in the environment and within your sample can rapidly degrade RNA resulting in low yield and poor quality. To avoid RNA degradation we suggest the following:

1. Have all equipment, reagents and disposable tips and tubes arranged and labeled prior to start working. We strongly encourage that all tubes are pre-labeled and if possible pre-aliquot reagents.
2. All materials coming in contact with the sample must be sterile and RNase-free. Use sterile disposable pipets, pipet tips and sterile disposable tubes whenever possible. Use sterile technique at all times. Clean all equipment and with 70% ethanol prepared with DEPC treated RNase-free deionized water.
3. Wear gloves during the entire procedure to avoid introducing RNase contaminants into the sample from your hands.
4. The 70% ethanol solution used for RNA isolation should be made with diethyl pyrocarbonate (DEPC) treated water. DEPC water is made by adding DEPC to a final concentration of 0.1%. Observe proper safety precautions (i.e. use a fume hood) when using DEPC, which is a powerful acylating agent and forms ethyl carbamate, a potent carcinogen, when exposed to ammonia. Note: DEPC in aqueous solutions hydrolyzes over time, even if refrigerated, or upon autoclaving or heating to 70° C for 1 h.
5. Reserve bench area, reagents, a set of pipets and possibly a centrifuge exclusively for RNA work. As discussed below, process tissue samples with Lysis Buffer or freeze tissue immediately after harvesting to avoid mRNA degradation.

RNA Yield	
Sample	**Average Yield**
Human Samples	
Whole blood	~2 μg/0.3 ml
Bone marrow	~3 μg/0.3 ml
Amniotic fluid	~2 μg/6 ml
Chorionic Villi	~2 μg/mg
Bodily Fluid (CSF, Semen, etc.)	~3 μg/300 μl
Buccal wash	~2 μg/10 ml
Cultured Cells, fibroblasts, amniocytes, lymphoblastiod cells	~10 μg/million cells
Solid Tissue* yield varies by tissue type	~1 μg/mg
Liver	~4 μg/mg
Lung	~1 μg/mg
Brain	~0.6 μg/mg
Heart	~1 μg/mg
Kidney	~3 μg/mg
Spleen	~3 μg/mg
Other Samples	
Plant tissue	1 μg/mg

Decontamination of Bodily Fluids and Tissue Samples

All human and animal samples used for purification of DNA & RNA should be considered infectious and proper decontamination protocol should be followed for eventual disposal. The following protocol is an easy and tested decontamination protocol.

Bodily Fluids

1. Prepare 1 L of 1x bleach solution in a large narrow mouth bottle. Keep the bottle capped. See recipe.
2. Transfer all liquid waste to this bottle. You can add up to 300 ml waste to this 1 L bleach solution.
3. At the end of the DNA purification protocol and after at least 1 hour decontamination, this bleach solution can be safely discarded in a regular sink/sewer. Precipitates appear after longer storage.
4. Let cold water run for 3-5 minutes to completely rinse, dilute and wash the sink.

Solid Waste

1. All solid wastes should be disposed of in orange biohazard bags for eventual autoclaving and disposal.
2. All sharps should be disposed in sharps container and disposed of after autoclaving.
3. Paper towels, pipet tips and disposable plastic ware should be treated as solid waste.

⚠ •All bodily fluids and tissue samples are to be considered infectious and hazardous.
•Wear gloves and protective clothing to prevent any exposure.
•All waste materials should be properly decontaminated and disposed following institutional guidelines.
•The decontamination protocol given here is for information only and is not a substitute for any other protocol established by your institution or OSHA.

ⓘ •Household bleach is a readily available and effective disinfectant.
•Common household bleach contains 5% sodium hypochlorite. This is a convenient 10X solution.
•Extended heating at 80°C to 100°C for 20 minutes or longer denatures and inactivates most pathogens.

Recipe

1x Bleach Solution	
Dilution of household bleach	
10x Bleach	Water
100 ml	900 ml

Size and MW of Various Nucleic Acids

Nucleic acid	Length in bases or base pairs	MW, Daltons
RNA		
tRNA (E.coli)	75	2.5×10^4
5S rRNA	120	3.6×10^4
16S rRNA	1700	5.5×10^5
18S rRNA	1900	6.1×10^5
23S rRNA	3700	1.2×10^6
28S rRNA	4800	1.6×10^6

Reference

1. *Ausubel, F.M., et al., Current Protocols in Molecular Biology, John Wiley and Sons, New York, 1988.*

Spectrophotometric Determination of RNA Concentration

Measuring the optical density (OD) or absorbance at 260 nm (A_{260}) in a UV spectrophotometer is a relatively accurate method for calculating the concentration of RNA in an aqueous solution if a standard curve is meticulously prepared. An A_{260} of 1.0, using a 1 cm path length, corresponds to a concentration of 50 µg/ml for double stranded DNA, 40 µg/ml for single stranded DNA and RNA, and 33 µg/ml for oligonucleotides. However, this method is not suitable for determining concentrations of dilute solutions, as the sensitivity of this method is not very high. For reliable readings, the concentration of nucleic acids must be greater than 1 µg/ml.

All dilutions and measurements should be done using RNase free pipets and solutions. The cuvettes should be RNase free also if the measured sample is to be recovered.

An example of the calculation involved in RNA quantification is shown below:

A_{260} = 1 = 40 µg/ml RNA

Volume of RNA sample = 50 µl
Dilution = 10 µl of RNA sample + 90 µl dH_20 (dilution factor is 10)
Measure A_{260} in a 1 ml cuvette: A_{260} = 0.25
Concentration of RNA stock = 40 µg/ml x A_{260} x dilution factor
= 40 µg/ml x 0.25 x 10
= 100 µg/ml
Total yield = concentration x volume of stock in ml
= 100 µg/ml x 0.05 ml
= 5 µg

Ordering Information

Omni-Pure™ RNA Purification Systems		
Product	**Catalog No.**	**Size***
Omni-Pure™ Blood & Bodily fluids RNA Spin Column Purification System	40-4080-05	50
Omni-Pure™ Blood & Bodily fluids RNA Spin Column Purification System	40-4080-10	100
Omni-Pure™ Blood & Bodily fluids RNA Spin Column Purification System	40-4080-50	500
Omni-Pure™ Universal RNA Spin Column Purification System	40-4090-05	50
Omni-Pure™ Universal RNA Spin Column Purification System	40-4090-10	100
Omni-Pure™ Universal RNA Spin Column Purification System	40-4090-50	500
Omni-Pure™ Blood & Bodily fluids RNA Purification System	40-4081-05	50
Omni-Pure™ Blood & Bodily fluids RNA Purification System	40-4081-10	100
Omni-Pure™ Blood & Bodily fluids RNA Purification System	40-4081-50	500
Omni-Pure™ Universal RNA Purification System	40-4091-05	50
Omni-Pure™ Universal RNA Purification System	40-4091-10	100
Omni-Pure™ Universal RNA Purification System	40-4091-50	500
Omni-Pure™ Viral RNA Spin Column Purification System	40-3650-01	100
Omni-Pure™ Viral RNA Spin Column Purification System	40-3650-05	500

**Unit of size is purification performed. Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

Omni-Pure™ Genomic DNA Purification Systems		
Product	**Catalog No.**	**Size***
Omni-Pure™ Blood DNA Purification System	40-4010-01	100
Omni-Pure™ Blood DNA Purification System	40-4010-05	500
Omni-Pure™ Blood DNA Purification System	40-4010-10	1000
Omni-Pure™ Tissue DNA Purification System	40-4050-01	100
Omni-Pure™ Tissue DNA Purification System	40-4050-05	500
Omni-Pure™ Tissue DNA Purification System	40-4050-10	1000
Omni-Pure™ Plant DNA Purification System	40-4060-01	100
Omni-Pure™ Plant DNA Purification System	40-4060-05	500
Omni-Pure™ Plant DNA Purification System	40-4060-10	1000
Omni-Pure™ Universal DNA Purification System	40-4070-01	100
Omni-Pure™ Universal DNA Purification System	40-4070-05	500
Omni-Pure™ Universal DNA Purification System	40-4070-10	1000

**Unit of size is purification performed. Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

Omni-Pure™ Viral & Microbial DNA & RNA Purification Systems		
Product	**Catalog No.**	**Size***
Omni-Pure™ Viral DNA Purification System	40-3720-05	50
Omni-Pure™ Viral DNA Purification System	40-3720-10	100
Omni-Pure™ Microbial DNA Purification System	40-3700-01	100
Omni-Pure™ Microbial DNA Purification System	40-3700-05	500
Omni-Pure™ Blood & Bodily fluids RNA Spin Column Purification System	40-4080-50	500
Omni-Pure™ Universal RNA Spin Column Purification System	40-4090-05	50
Omni-Pure™ Universal RNA Spin Column Purification System	40-4090-10	100
Omni-Pure™ Universal RNA Spin Column Purification System	40-4090-50	500
Omni-Pure™ Blood & Bodily fluids RNA Purification System	40-4081-05	50
Omni-Pure™ Blood & Bodily fluids RNA Purification System	40-4081-10	100
Omni-Pure™ Blood & Bodily fluids RNA Purification System	40-4081-50	500
Omni-Pure™ Universal RNA Purification System	40-4091-05	50
Omni-Pure™ Universal RNA Purification System	40-4091-10	100
Omni-Pure™ Universal RNA Purification System	40-4091-50	500
Omni-Pure™ Viral RNA Spin Column Purification System	40-3650-01	100
Omni-Pure™ Viral RNA Spin Column Purification System	40-3650-05	500

**Unit of size is purification performed. Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

Omni-Clean™ Gel DNA Purification and Concentration Systems		
Product	**Catalog No.**	**Size***
Omni-Clean™ Gel DNA Beads Purification System	40-4110-10	100
Omni-Clean™ Gel DNA Beads Purification System	40-4110-50	500
Omni-Clean™ Gel DNA Spin Column Purification System	40-4120-10	100
Omni-Clean™ Gel DNA Spin Column Purification System	40-4120-50	500
Omni-Clean™ DNA Beads Concentration System	40-4130-10	100
Omni-Clean™ DNA Beads Concentration System	40-4130-50	500
Omni-Clean™ DNA Spin Column Concentration System	40-4140-10	100
Omni-Clean™ DNA Spin Column Concentration System	40-4140-50	500

* *Unit of size is purification performed. Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

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