User Guide

PicoPure™ RNA Isolation Kit

Catalog # KIT0202, KIT0204

ARCTURUS

Version D
For Research Use Only

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I. Introduction

A. Background

The PicoPure™ RNA Isolation Kit enables researchers to recover total cellular RNA from pico-scale samples. The PicoPure RNA Isolation Kit is optimized for use with cells acquired using Laser Capture Microdissection (LCM) on CapSure®HS LCM Caps and CapSure® Macro LCM Caps but can be used with various cell samples to isolate total cellular RNA. Researchers can obtain high recoveries of total cellular RNA from as little as a single cell to samples with up to 100 μg of RNA. Total cellular RNA isolated using the PicoPure RNA Isolation Kit produces RNA in a small volume of low ionic strength buffer, ready for use in downstream applications including reverse transcription for Q-PCR, production of labeled cDNA, or linear amplification using the RiboAmp® RNA Amplification Kit.

The PicoPure RNA Isolation Kit contains RNA extraction and purification reagents, RNA purification columns, and this User Guide.

B. Performance Specifications

RNA isolated using the PicoPure Kit is intact and ready for downstream applications when starting with samples with high RNA quality. RT-PCR of GAPDH from isolated total RNA from 100 LCM captured cells prepared using the HistoGene™ LCM Frozen Section Staining Kit produces visible product upon gel electrophoresis separation and staining.

Researchers can complete total cellular RNA isolations from cells captured on CapSure HS LCM Caps or CapSure Macro LCM Caps in less than an hour. RNA extraction from LCM caps requires 30 minutes of incubation time, and the RNA purification process takes less than 20 minutes.

C. Quality Control

Arcturus performs functional testing on the PicoPure RNA Isolation Kit using all kit components and the protocol described in this user guide. Using the kit, Quality Control Specialists isolate RNA from 100 cells acquired by LCM and analyze it by RT-PCR using primers to generate amplicons of a specific gene or gene fragment. The reaction product is separated by electrophoresis and stained. A single amplicon band of the correct size is visualized.

RNA purification columns are tested by lot to confirm the absence of nucleic acids and nuclease activity. Column nucleic acid binding and recovery performance must meet quality standards.

A Quality Control Certificate included with each shipment describes the tests performed and includes individual lot testing data.

D. Storage and Stability

Store the PicoPure RNA Isolation Kit at room temperature. Properly stored kits are stable until the expiration date indicated on the package.

E. Material Safety and Data Sheets

Material Safety and Data Sheets (MSDS) for kit chemical components are available from Arcturus Technical Services. Call 888.446.7911 or 650.962.3020, send e-mail to techsupport@arctur.com, or download the file from www.arctur.com.

F. Related Products from Arcturus

CapSure® HS LCM Caps
CapSure HS LCM Caps enable the highest specificity cell capture from LCM. CapSure HS LCM Caps come with PrepStrip™ Tissue Preparation Strips and ExtracSure™ Sample Extraction Devices.

CapSure® Macro LCM Caps
CapSure Macro LCM Caps enable large quantity LCM captures. Each box of caps also contains PrepStrip Tissue Preparation Strips.

HistoGene™ LCM Frozen Section Staining Kit
The HistoGene LCM Frozen Section Staining Kit simplifies tissue staining and dehydration while maintaining RNA integrity. The HistoGene Kit uses an optimized tissue section processing system that includes reagents and protocols for preserving tissue and cellular RNA to maximize quality and yield of RNA from cells acquired by LCM.

HistoGene™ LCM Immunofluorescence Staining Kit
The HistoGene LCM Immunofluorescence Staining Kit is the only kit designed to enable retrieval of high-quality RNA from immunofluorescently stained frozen tissue. It enables convenient and reliable staining, dehydration and LCM of tissue sections with protocols streamlined and optimized both for optimal LCM captures and maintaining RNA quality for downstream applications that require intact RNA, like microarray analysis and RT-PCR.

RiboAmp® RNA Amplification Kit
The RiboAmp RNA Amplification Kit enables the production of microgram quantities of antisense RNA (aRNA) from nanogram quantities of total cellular RNA. Amplified RNA produced using the kit is suitable for labeling and use for probing expression microarrays. The kit achieves amplifications of up to 1000-fold in one round of amplification, and amplifications of up to 1,000,000-fold in two rounds. The RiboAmp Kit comes with all necessary enzymes, reagents, and nucleic acid purification devices needed to complete the included amplification protocol.

RiboAmp® HS RNA Amplification Kit
The RiboAmp HS RNA Amplification Kit starts with picogram total cellular RNA input and enables the production of microgram quantities of antisense RNA (aRNA). The kit provides the greatest level of sensitivity in starting RNA quantities to produce enough RNA suitable for labeling and hybridizing onto expression microarrays. The RiboAmp HS Kit come with all necessary enzymes, reagents, and MiraCol Purification Columns needed to complete the included amplification protocol.

RiboAmp® OA RNA Amplification Kit
The RiboAmp OA RNA Amplification Kit enables the production of microgram quantities of antisense RNA (aRNA) from nanogram quantities of total cellular RNA. This specially formulated kit conveniently prepares small samples for direct labeling to hybridize onto oligonucleotide arrays requiring labeled aRNA, such as GeneChip® Probe Arrays (Affymetrix). Also, the Kit may be used for preparation of labeled aRNA to hybridize onto cDNA platforms while eliminating the need for post-amplification labeling. The RiboAmp OA Kit is coupled with commercially available transcript labeling systems that incorporate either biotin or fluorescent labels directly into aRNA.

Paradise™ Reagent System
The Paradise Reagent System is the only reagent system desinged to enable gene expression studies using formalin-fixed paraffin-embedded (FFPE) tissue samples. Components include sample preparation and staining reagents, RNA extraction and isolation reagents, RNA amplification reagents and a comprehensive user guide.

II. Kit Components

A. Reagents and Supplies Provided

The PicoPure RNA Isolation Kit comes with the following items:

Item	Vial Name
Conditioning Buffer	CB
Extraction Buffer	XB
70% Ethanol	EtOH
Wash Buffer 1	W1
Wash Buffer 2	W2
Elution Buffer	EB
RNA purification columns with collection tubes	
Microcentrifuge tubes	

III. Preliminary Steps

A. Recommendations for RNase-free Technique

RNase contamination will cause experimental failure. Minimize RNase contamination by adhering to the following recommendations throughout your experiment:

- Always handle RNA in a manner that avoids introduction of RNases.
- Wear disposable gloves and change them frequently to prevent the introduction of RNases from skin surfaces.
- After putting on gloves, avoid touching surfaces that may introduce RNases onto glove surfaces.
- Do not use reagents not supplied in the PicoPure RNA Isolation Kit. Substitution of reagents or kit components may adversely affect yields or introduce RNases.
- Use only new plasticware that is certified nucleic acid-free.
- Use only new, sterile, RNase-free pipette tips and micro-centrifuge tubes.
- Clean work surfaces with commercially available RNase decontamination solutions prior to performing reactions.

B. Recommendations for Storing RNA

Begin the PicoPure RNA Isolation Kit protocol immediately following acquisition of cells by LCM. The Extraction Buffer (XB) stabilizes RNA. Cell extracts resulting from completion of Part I of the protocol may be stored at –80°C.

Following protocol completion, use isolated RNA immediately for amplification, or store at –80°C for up to 6 months.

C. Additional Lab Equipment and Materials Required

Ensure that you have ready access to the following laboratory equipment and materials before you begin. These items are not included in the PicoPure RNA Isolation Kit:

1. **Equipment**
- ❑ Microcentrifuge (Eppendorf 5415D or similar)
- ❑ 2–20 µl pipettor
- ❑ 20–200 µl pipettor
- ❑ Incubation oven

2. **Materials**
- ❑ Nuclease-free pipette tips
- ❑ 0.5 ml microcentrifuge tubes (Applied BioSystems Catalog #N8010611)
- ❑ 2 ml lidless tubes (PGC Scientific, Catalog #16-8101-06)

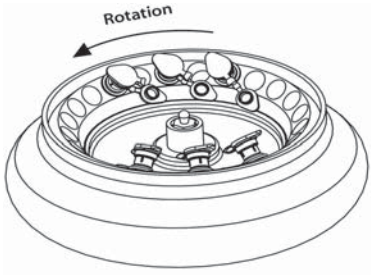
3. **Reagents (optional)**
- ❑ DNase, RNase-free (see Appendix)

D. DNase Treatment of Samples

The enhanced sensitivity of the PicoPure RNA Isolation Kit is made possible by the use of proprietary components. Some of these components may interfere with techniques to assess RNA quality such as gel electrophoresis or using the Agilent 2100 BioAnalyzer. In order to prevent these artifacts as well as genomic DNA contamination, we strongly recommend DNase treatment. In addition, if performing reverse transcription or amplification using RiboAmp RNA amplification Kits after RNA isolation, DNase treatment is recommended. Please see Appendix A for a detailed protocol.

E. Nucleic Acid Elution Using Spin Columns

Spin columns and 0.5 ml microcentrifuge tubes are provided for nucleic acid elution. Improper orientation of tubes, with caps open during centrifugation, may result in cap breakage or sample loss. To correctly use the column-tube assembly, insert a spin column into the 0.5 ml tube, aligning the two cap hinges as illustrated. Load Elution Buffer onto the column and incubate as directed. Place the column-tube assembly into a 2 ml lidless support tube (PGC Scientific, Catalog #16-8101-06) in the centrifuge rotor. Skip one rotor position between assemblies, and position assemblies with the 0.5 ml tube cap trailing the tube during centrifugation as shown. (Check for a mark on the centrifuge indicating rotation direction.) Centrifuge as directed in the protocol.



IV. Protocol

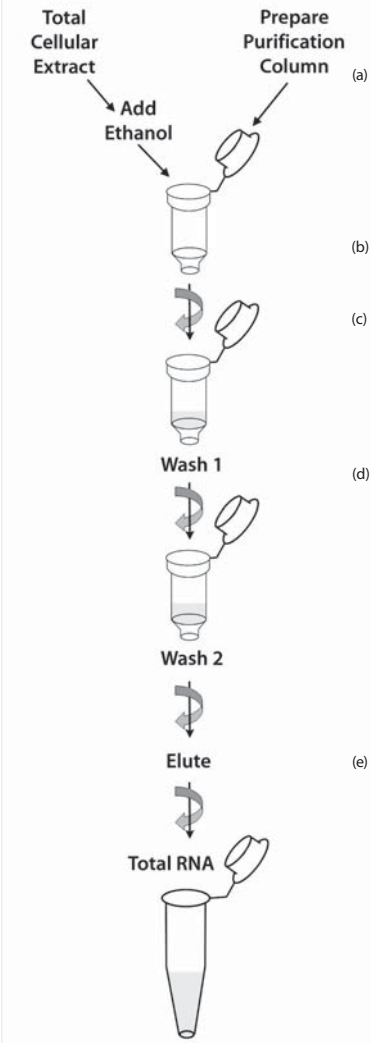
A. Overview

The flow chart illustrates the PicoPure RNA Isolation Kit procedure: (a) Extract RNA from a CapSure HS LCM Cap or a CapSure Macro LCM Cap. (b) Load cell extract onto a pre-conditioned purification column. (c) Spin the extract through the column to capture RNA on the purification column membrane. (d) Wash the column twice with wash buffer, and (e) elute the RNA in low ionic strength buffer. The entire process, including incubations, can be completed in less than an hour, and the isolated total cellular RNA is ready for use in downstream applications.

PicoPure is capable of isolating extremely small amounts of RNA. It is important not to introduce nucleic acid contamination.

If you used CapSure HS LCM Caps to capture cells for RNA isolation, use the following protocol presented under heading B. If you used Capsure Macro LCM Caps to capture cells, use the protocol presented under C on page 14. For RNA isolation of cell pellets, use the protocol presented in Appendix B on page 20.

The PicoPure RNA Isolation Process



B. Protocol for Use with CapSure HS LCM Caps

1. RNA Extraction

a. Dispense Extraction Buffer (XB) and incubate:

i. Capture cells and assemble the CapSure HS Cap with the ExtracSure Extraction Device. Refer to the CapSure HS Caps User Guide for complete instructions.

ii. Place the CapSure–ExtracSure assembly in a CapSure HS Alignment Tray and pipette 10 µL Extraction Buffer (XB) into the buffer well.

iii. Place a new 0.5 mL microcentrifuge tube (Applied BioSystems Catalog # N8010611) onto the CapSure–ExtracSure assembly. Cover with Incubation Block preheated to 42°C.

iv. Incubate assembly for 30 minutes at 42°C.

b. Centrifuge the microcentrifuge tube with the CapSure–ExtracSure assembly at 800 x *g* for two minutes to collect cell extract into the microcentrifuge tube.

c. After centrifugation, the microcentrifuge tube contains the cell extract required to complete the protocol. Remove the microcentrifuge tube from the CapSure–ExtracSure assembly and save the microcentrifuge tube with the cell extract in it.

d. Proceed with RNA isolation protocol or freeze cell extract at –80°C.

OK *It is okay to stop at this point in the protocol.*

2. RNA Isolation

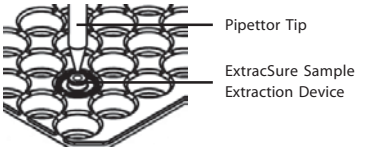
a. Pre-condition the RNA Purification Column:

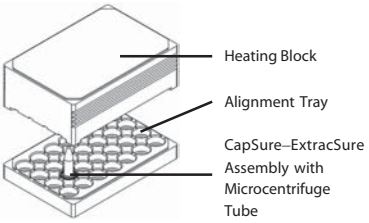
i. Pipette 250 µL Conditioning Buffer (CB) onto the purification column filter membrane.

ii. Incubate the RNA Purification Column with Conditioning Buffer for 5 minutes at room temperature.

iii. Centrifuge the purification column in the provided collection tube at 16,000 x *g* for one minute.

⚠ *Prior to use, mix Extraction Buffer (XB) thoroughly. Extraction Buffer (XB) may form precipitate upon storage. Dissolve precipitate prior to use by mixing thoroughly. If necessary, warm the XB vial to redissolve Extraction Buffer prior to use.*





⚠ *The CapSure HS LCM Caps and the ExtracSure Device are compatible with 0.5 ml thin-walled reaction tubes from Applied BioSystems. These reaction tubes should be used for RNA extraction.*

b. Pipette 10 µL of 70% Ethanol (EtOH) into the cell extract from Part 1 (RNA Extraction). Mix well by pipetting up and down. DO NOT CENTRIFUGE.

c. Pipette the cell extract and EtOH mixture into the pre-conditioned purification column. The cell extract and EtOH will have a combined volume of approximately 20 µL.

d. To bind RNA, centrifuge for 2 minutes at 100 x *g*, immediately followed by a centrifugation at 16,000 x *g* for 30 seconds to remove flowthrough.

e. Pipette 100 µL Wash Buffer 1 (W1) into the purification column and centrifuge for one minute at 8,000 x *g*.

Optional: DNA may be removed by DNase treatment from the preparation at this point. See Appendix A for a detailed protocol.

f. Pipette 100 µL Wash Buffer 2 (W2) into the purification column and centrifuge for one minute at 8,000 x *g*.

g. Pipette another 100 µL Wash Buffer 2 (W2) into the purification column and centrifuge for two minutes at 16,000 x *g*. Check the purification column for any residual wash buffer. If wash buffer remains re-centrifuge at 16,000 x *g* for one minute.

h. Transfer the purification column to a new 0.5 mL microcentrifuge tube provided in the kit.

i. Pipette Elution Buffer (EB) directly onto the membrane of the purification column (Gently touch the tip of the pipette to the surface of the membrane while dispensing the elution buffer to ensure maximum absorption of EB into the membrane). Use the Elution Volume Guide table to select the correct volume of Elution Buffer to use in this step.

Elution Volume Guide	
Recommended Volume	11 µL
Maximum Volume	30 µL

The eluted RNA can be used directly in the RiboAmp RNA Amplification Kit or in reverse transcription protocols. For samples eluted in the maximum volume, the eluted RNA may be concentrated in a vacuum centrifuge to reduce the volume to 10 µL.

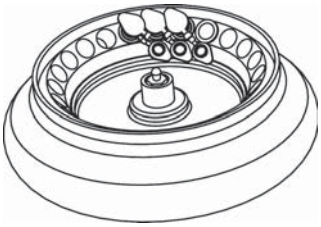
⚠ *Flowthrough waste following centrifugation is usually present as only a small volume, and therefore it is not necessary to discard the flowthrough waste after every centrifugation step. Make sure that the accumulated flowthrough waste does not make contact with the purification column. Flowthrough waste should be discarded when the waste fluid level approaches the surface of the purification column.*

⚠ *DNase treatment is recommended if performing reverse transcription or amplification with the RiboAmp RNA Amplification Kit after RNA isolation.*

⚠ *Remove all traces of wash buffer prior to transferring purification column to the new microcentrifuge tube. To remove wash buffer, discard flow-through waste and recentrifuge the column for one minute at 16,000 x g.*

j. Incubate the column for one minute at room temperature.

k. Centrifuge the column for one minute at 1,000 x *g* to distribute EB in the column, and then spin for one minute at 16,000 x *g* to elute RNA. The isolated RNA is now ready for use in downstream applications such as reverse transcription or amplification with the RiboAmp RNA Amplification Kit. The entire sample may be used immediately or stored at –80°C until use.



⚠ *To avoid potential breakage of the microcentrifuge tube cap during centrifugation, insert the purification column/ 0.5 mL tube assembly into a lidless 1.7/2.0 mL tube. Insert this assembly into adjacent rotor holes as illustrated. Rest the tube cap against the tube immediately clockwise to it. Place an empty, lidless 1.7/2.0 mL tube into the rotor hole adjacent in the clockwise direction to the last assembly.*

⚠ *Quantitation of isolated RNA through UV spectrophotometry or measurement by fluorescence based methods may not be possible for samples containing less than 1 µg of total RNA. Measurements will be affected by components in the eluted sample, which cause an overestimation of the total RNA content.*

C. Protocol for Use with CapSure Macro LCM Caps

1. RNA Extraction

Prior to use, mix Extraction Buffer (XB) thoroughly. Extraction Buffer (XB) may form precipitate upon storage. Dissolve precipitate prior to use by mixing thoroughly. If necessary, warm the XB vial to redissolve Extraction Buffer prior to use.

a. Dispense Extraction Buffer (XB) and incubate:

   i. Pipette 50 µL Extraction Buffer (XB) into a 0.5 mL microcentrifuge tube (Applied BioSystems Catalog # N8010611).

   ii. Insert CapSure Macro LCM Cap onto the microcentrifuge tube using an LCM Cap Insertion Tool.

   iii. Invert the CapSure Cap–microcentrifuge tube assembly. Tap the microcentrifuge tube to ensure all Extraction Buffer (XB) is covering the CapSure Macro LCM Cap.

   iv. Incubate assembly for 30 minutes at 42°C.

b. Centrifuge assembly at 800 x *g* for two minutes to collect cell extract into the microcentrifuge tube.

c. After centrifugation, the microcentrifuge tube contains the cell extract required to complete the protocol. Remove the CapSure Macro LCM Cap and save the microcentrifuge tube with the cell extract in it.

d. Proceed with RNA isolation protocol or freeze cell extract at –80°C.

OK *It is okay to stop at this point in the protocol.*

2. RNA Isolation

a. Pre-condition the RNA Purification Column:

   i. Pipette 250 µL Conditioning Buffer (CB) onto the purification column filter membrane.

   ii. Incubate the RNA Purification Column with Conditioning Buffer for 5 minutes at room temperature.

   iii. Centrifuge the purification column in the provided collection tube at 16,000 x *g* for one minute.

Flowthrough waste following centrifugation is usually present as only a small volume, and therefore it is not necessary to discard the flowthrough waste after every centrifugation step. Make sure that the accumulated flow-through waste does not make contact with the purification column. Flow-through waste should be discarded when the waste fluid level approaches the surface of the purification column.

b. Pipette 50 µL of 70% Ethanol (EtOH) into the cell extract from Part 1 (RNA Extraction). Mix well by pipetting up and down. DO NOT CENTRIFUGE.

c. Pipette the cell extract and EtOH mixture into the pre-conditioned purification column. The cell extract and EtOH will have a combined volume of approximately 100 µL.

d. To bind RNA to the column, centrifuge for 2 minutes at 100 x *g*, immediately followed by a centrifugation at 16,000 x *g* for 30 seconds to remove flowthrough.

e. Pipette 100 µL Wash Buffer (W1) into the purification column and centrifuge for one minute at 8,000 x *g*.

   Optional: DNA may be removed by DNase treatment from the preparation at this point. See Appendix A for a detailed protocol.

f. Pipette 100 µL Wash Buffer 2 (W2) into the purification column and centrifuge for one minute at 8,000 x *g*.

g. Pipette another 100 µL Wash Buffer (W2) into the purification column and centrifuge for two minutes at 16,000 x *g*. Check the purification column for any residual wash buffer. If wash buffer remains re-centrifuge at 16,000 x *g* for one minute.

h. Transfer the purification column to a new 0.5 mL microcentrifuge tube provided in the kit.

i. Pipette Elution Buffer (EB) directly onto the membrane of the purification column (Gently touch the tip of the pipette to the surface of the membrane while dispensing the elution buffer to ensure maximum absorption of EB into the membrane). Use the Elution Volume Guide table to select the correct volume of the Elution Buffer to use in this step.

DNase treatment is recommended if performing reverse transcription or amplification with the RiboAmp RNA Amplification Kit after RNA isolation.

Remove all traces of wash buffer prior to transferring purification column to the new microcentrifuge tube. To remove wash buffer, discard flow-through waste and recentrifuge the column for one minute at 16,000 x g.

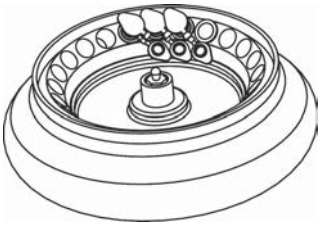
Elution Volume Guide

Recommended Volume	11 µL
Maximum Volume	30 µL

The eluted RNA can be used directly in the RiboAmp RNA Amplification Kit or in reverse transcription protocols. For samples eluted in the maximum volume, the eluted RNA may be concentrated in a vacuum centrifuge to reduce the volume to 10 µL.

j. Incubate the purification column for one minute at room temperature.

k. Centrifuge the column for one minute at 1,000 x *g* to distribute EB in the column, then for one minute at 16,000 x *g* to elute RNA. The isolated RNA is now ready for use in downstream applications such as reverse transcription or amplification with the RiboAmp Kit. The entire sample may be used immediately or stored at –80°C until use.



⚠ *To avoid potential breakage of the microcentrifuge tube cap during centrifugation, insert the purification column/ 0.5 mL tube assembly into a lidless 1.7/2.0 mL tube. Insert this assembly into adjacent rotor holes as illustrated. Rest the tube cap against the tube immediately clockwise to it. Place an empty, lidless 1.7/2.0 mL tube into the rotor hole adjacent in the clockwise direction to the last assembly.*

⚠ *Quantitation of isolated RNA through UV spectrophotometry or measurement by fluorescence based methods may not be possible for samples containing less than 1 μg of total RNA. Measurements will be affected by components in the eluted sample, which cause an overestimation of the total RNA content.*

V. Troubleshooting

A. Isolated RNA is of Poor Quality

1. Verify quality of source tissue of LCM cells. The greatest factor affecting the quality of isolated RNA is the integrity of the RNA in the original tissue sample. RNA degradation due to RNase activity occurs rapidly, especially upon tissue removal such as through biopsy and needle aspiration. Tissue should be embedded and frozen immediately upon removal to reduce RNase activity. For suggestions on verifying quality, please call Arcturus Technical Support.
2. Use the HistoGene LCM Frozen Section Staining Kit to prepare slides for LCM. Specialized staining protocols and reagents are required for optimal RNA preservation in LCM samples. Arcturus has developed and validated the HistoGene LCM Frozen Section Staining Kit for preparing and staining tissues for LCM while maintaining RNA integrity.
3. Perform LCM immediately after preparing LCM slides. LCM sample slides are dehydrated in the final step of preparation, so RNase activity is minimized. However, the risk of moisture and RNases entering the sample following preparation increases with the interval of time between slide preparation and RNA isolation.
4. Pipette PicoPure Extraction Buffer (XB) onto cells acquired by LCM immediately after cell capture. Extraction Buffer (XB) stabilizes RNA by denaturing nucleases. Complete Part 1 (RNA Extraction) of the PicoPure RNA Isolation Kit Protocol without stopping. The cell extract resulting from the completion of Part I (RNA Extraction) may be stored in Extraction Buffer (XB) at –80°C.
5. Use only frozen or alcohol-fixed tissue or cytosmeared cells. Using tissue or cell samples that have been subjected to cross-linking fixative such as formalin or paraformaldehyde diminishes RNA quality. PicoPure Extraction Buffer (XB) is not suitable for extraction of RNA from cross-linked tissue. For isolation of RNA from formalin-fixed, paraffin-embedded (FFPE) samples, use the Paradise Reagent System.

B. RNA Yield is Low

1. RNA integrity has been compromised. Verify quality of initial tissue sample or LCM slide (see A.1). Poor quality RNA may not bind effectively to the purification column membrane, decreasing overall RNA yield.
2. Buffer concentrations in extraction mixtures are incorrect due to inadequate mixing with 70% Ethanol. Ensure all buffers are completely mixed and all solids are dissolved prior to use.
3. Elution Buffer (EB) concentration is incorrect due to contamination with Wash Buffer 2 (W2). Ensure that all Wash Buffer 2 has been removed by centrifugation before proceeding to add Elution Buffer. Residual Wash Buffer 2 (W2) on the purification column filter membrane will alter the concentration of Elution Buffer (EB), resulting in poor RNA elution. If any Wash Buffer 2 (W2) remains in or on the purification column, recentrifuge it to remove the residual buffer before proceeding to elution.
4. Extraction step incubation was too short. Incubate the LCM sample in Extraction Buffer (XB) for a full 30 minutes at 42°C. Complete cell extraction from fixed, dehydrated samples requires this validated incubation condition.

VI. Appendix

A. DNase Treatment

DNase treatment may be performed directly within the purification column when downstream applications require removal of genomic DNA to reduce risk of DNA interference.. The following protocol utilizing the RNase-Free DNase Set (Qiagen, catalog#79254) may be used.

Protocol is applied during Part 2, RNA Isolation protocol between Steps e and f.

1. Pipette 5 µL DNase I Stock Solution to 35 µL Buffer RDD (provided with RNase-Free DNase Set). Mix by gently inverting. For isolations from larger samples such as cell pellets (see Appendix B), pipette 10 µl DNase I stock solution to 30 µL Buffer RDD.
2. Pipette the 40 µL DNase incubation mix directly into the purification column membrane. Incubate at room temperature for 15 minutes.
3. Pipette 40 µL PicoPure RNA Kit Wash Buffer 1 (W1) into the purification column membrane. Centrifuge at 8000 x *g* for 15 seconds.
4. Proceed with RNA Isolation protocol Step f of Section IV, B2 or Section IV C2.

B. RNA Extraction from Cell Pellets

The PicoPure RNA Isolation Kit may be used for non-LCM samples such as cell sample cultures in suspension[1]. The following is a suggested protocol for the treatment of cell culture cells in suspension. Adherent cells need to be detached from the cell culture flask or disk prior to preceeding.

The protocol replaces Part 1:RNA Extraction of either protocol in Chapter IV.

1. Pellet cells in a microcentrifuge tube by centrifuging at 3,000 *x g* for 10 minutes.
2. Properly dispose of the supernatant.
3. Resuspend the cell pellet in one mL of cell suspension media (0.9 mL of 1 x PBS/10%BSA; 0.1 mL of 0.5 M EDTA). DO NOT VORTEX.
4. Centrifuge the cell suspension at 3,000 x *g* for five minutes.
5. Properly dispose of supernatant.
6. Extract the cells with 100 µL of Extraction Buffer (XB). Resuspend the cell pellet gently by pipetting. DO NOT VORTEX.
7. Incubate at 42° for 30 minutes.
8. Centrifuge the sample at 3,000 x *g* for two minutes.
9. Pipette the supernatant containing the extracted RNA into a new microcentrifuge tube, avoiding pick-up of pelleted material.
10. Proceed with RNA Isolation or freeze at –70° C.
11. Proceed with the RNA Isolation protocol in sections IV:B or IV:C with the following modification to step b:

    Pipette 100 µL of 70% Ethanol (EtOH) to the cell extract (or equal volume to the cell extract). Mix well by pipetting up and down. DO NOT CENTRIFUGE. The combined volume will be approximately 200 µL.

[1]The PicoPure RNA Isolation Kit is not recommended for RNA isolation from whole blood, plant, or fungal samples. The addition of whole tissues and cells (e.g. whole blood samples) directly onto the purification columns is not recommended.

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