applied biosystems

PicoPure™ RNA Isolation Kit

USER GUIDE

Catalog Numbers KIT0204 and KIT0214

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Revision A

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN1000651 A (English)

Revision	Date	Description
A	10 September 2024	• Updated quality control, trademarks, format, style, and branding. • Minor edits for clarification.

The information in this guide is subject to change without notice.

Contents

1 Product information

Background

The Arcturus™ 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 qPCR, production of labeled cDNA, or linear amplification using a RiboAmp™ RNA Amplification Kit.

The PicoPure™ RNA Isolation Kit contains RNA extraction and purification reagents, and RNA purification columns.

Performance specifications

RNA isolated using the PicoPure™ RNA Isolation 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.

Quality control

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

Contents and storage

The components included with the PicoPure™ RNA Isolation Kit (Cat. No. KIT0204, 40 samples; Cat. No. KIT0214, 200 samples) are listed in the following table. Properly stored kits are stable until the expiration date indicated on the package.

Components	Vial name	Storage[1]
Conditioning Buffer	CB	15°C to 30°C
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		

[1] Store all components at room temperature.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Equipment	
Microcentrifuge (Eppendorf™ 5415D or similar)	**MLS**
2–20 µL pipettor	**MLS**
20–200 µL pipettor	**MLS**
Incubation oven	**MLS**
Materials	
Nuclease-free pipette tips	**MLS**
GeneAmp™ Thin-Walled Reaction Tube, with domed cap, 0.5 mL (or equivalent)	N8010611
Fisherbrand™ Rimmed Microcentrifuge Tubes, 2 mL lidless (or equivalent)	02-681-453
(Optional) **Reagents**	
QIAGEN™ RNase-Free DNase Set (or equivalent)	79254

Related products

Catalog numbers that appear as links open the web pages for those products.

Item	Quantity	Cat. No.
CapSure™ HS LCM Caps	32 caps 160 caps	LCM0214 LCM0215
CapSure™ Macro LCM Caps	48 caps 240 caps	LCM0211 LCM0212
HistoGene™ LCM Frozen Section Staining Kit	72 applications	KIT0401
HistoGene™ LCM Immunofluorescence Staining Kit	32 applications	KIT0420
Arcturus™ RiboAmp™ PLUS RNA Amplification Kit	6 samples 24 samples	KIT0521 KIT0501
Arcturus™ RiboAmp™ HS PLUS RNA Amplification Kit	6 samples 24 samples	KIT0525 KIT0505
Arcturus™ Paradise™ PLUS RNA Extraction and Isolation Kit	12 samples	KIT0312I

2 Before you begin

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 microcentrifuge tubes.
- Clean work surfaces with commercially available RNase decontamination solutions prior to performing reactions.

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 RNA extraction may be stored at –80°C.

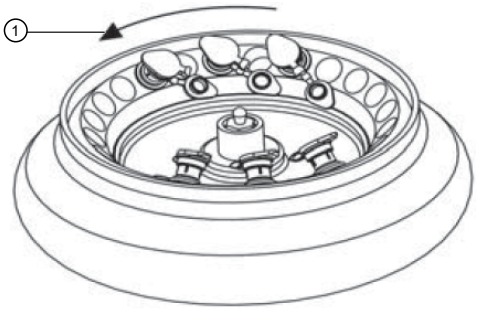
Following protocol completion, use isolated RNA immediately for amplification, or store at –80°C for up to 6 months.

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™ Instrument. 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 a RiboAmp™ RNA Amplification Kit after RNA isolation, DNase treatment is recommended. For a detailed protocol, see “DNase treatment” on page 19.

Nucleic acid elution using RNA purification columns

RNA purification 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 RNA purification column into the 0.5 mL tube, aligning the two cap hinges as illustrated. Load Elution Buffer (EB) onto the RNA purification column and incubate as directed. Place the column-tube assembly into a 2 mL lidless support tube 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 ①. *(Optional)* Place an empty, lidless 2 mL tube into the rotor hole adjacent in the clockwise direction to the last assembly (not shown). Centrifuge as directed in the protocol.



① Rotation direction

3 PicoPure™ RNA Isolation Kit protocol

The workflow illustrates the PicoPure™ RNA Isolation Kit procedure. The entire process, including incubations, can be completed in less than an hour. 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, see “Protocol for use with CapSure™ HS LCM Caps” on page 11.

If you used CapSure™ Macro LCM Caps to capture cells, see “Protocol for use with CapSure™ Macro LCM Caps” on page 14.

For RNA isolation from cells, see “RNA extraction from cell pellets” on page 19.

PicoPure™ RNA Isolation Kit workflow

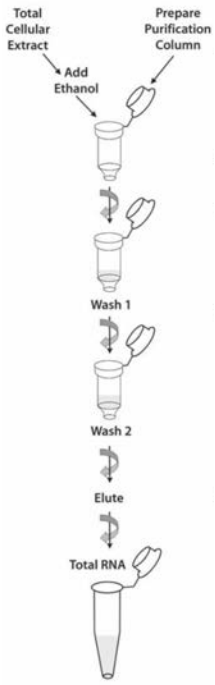
Extract RNA from a CapSure™ HS LCM Cap (page 11) **or a CapSure™ Macro LCM Cap** (page 14)

Load cell extract onto a preconditioned RNA purification column

Centrifuge the extract through the RNA purification column to capture RNA on the column membrane

Wash the RNA purification column twice with wash buffer

Elute the RNA in low ionic strength buffer



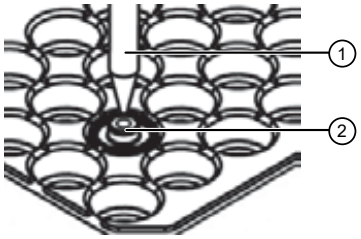
Protocol for use with CapSure™ HS LCM Caps

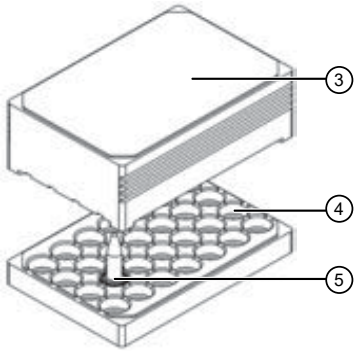
RNA extraction (CapSure™ HS LCM Caps)

IMPORTANT! Prior to use, mix Extraction Buffer (XB) thoroughly. Extraction Buffer may form precipitate upon storage. Dissolve precipitate prior to use by mixing thoroughly. If necessary, warm the XB vial at 42°C to redissolve Extraction Buffer prior to use.

Note: The CapSure™ HS LCM Caps and the ExtracSure™ Sample Extraction Device are compatible with 0.5 mL thin-walled reaction tubes (Cat. No. N8010611). These microcentrifuge tubes should be used for RNA extraction.

1. Dispense Extraction Buffer (XB) and incubate.
   a. Capture cells and assemble the CapSure™ HS LCM Cap with the ExtracSure™ Sample Extraction Device ②.
   b. Place the CapSure™–ExtracSure™ assembly in a CapSure™ HS Alignment Tray ④ and pipet 10 µL Extraction Buffer (XB) into the buffer well.
   c. Place a new 0.5 mL microcentrifuge tube onto the CapSure™–ExtracSure™ assembly ⑤. Cover with incubation block ③ preheated to 42°C.
   d. Incubate assembly for 30 minutes at 42°C.
2. Centrifuge the microcentrifuge tube with the CapSure™–ExtracSure™ assembly at 800 × *g* for 2 minutes to collect cell extract into the microcentrifuge tube.
   After centrifugation, the microcentrifuge tube contains the cell extract required to complete the protocol.
3. Remove the microcentrifuge tube from the CapSure™–ExtracSure™ assembly and save the microcentrifuge tube with the cell extract in it.





① Pipettor tip
② ExtracSure™ Sample Extraction Device
③ Heating block
④ CapSure™ HS Alignment Tray
⑤ CapSure™–ExtracSure™ assembly with microcentrifuge tube

Proceed with RNA isolation protocol (see “RNA isolation (CapSure™ HS LCM Caps)” on page 12).

STOPPING POINT The cell extract can be stored frozen at –80°C for up to 6 months.

RNA isolation (CapSure™ HS LCM Caps)

1. Pre-condition the RNA purification column.
   a. Pipet 250 µL Conditioning Buffer (CB) onto the RNA purification column filter membrane.
   b. Incubate the RNA purification column with Conditioning Buffer (CB) for 5 minutes at room temperature.
   c. Centrifuge the RNA purification column in the provided collection tube at 16,000 × *g* for 1 minute.
2. Pipet 10 µL of 70% Ethanol (EtOH) into the cell extract from RNA extraction (see "RNA extraction (CapSure™ HS LCM Caps)" on page 11), then mix well by pipetting up and down.

   IMPORTANT! Do not vortex.
3. Pipet the cell extract and EtOH mixture into the preconditioned RNA purification column.

   The cell extract and EtOH will have a combined volume of approximately 20 µL.
4. To bind RNA, centrifuge for 2 minutes at 100 × *g*, immediately followed by a centrifugation at 16,000 × *g* for 30 seconds to remove flowthrough.

   Note: 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. Ensure that the accumulated flowthrough waste does not make contact with the RNA purification column. Flowthrough waste should be discarded when the waste fluid level approaches the surface of the RNA purification column.
5. Pipet 100 µL Wash Buffer 1 (W1) into the RNA purification column, then centrifuge for 1 minute at 8,000 × *g*.

   (Optional) DNA may be removed by DNase treatment from the preparation at this step. For a detailed protocol, see "DNase treatment" on page 19.

   Note: DNase treatment is recommended if performing reverse transcription or amplification with a RiboAmp™ RNA Amplification Kit after RNA isolation.
6. Pipet 100 µL Wash Buffer 2 (W2) into the RNA purification column, then centrifuge for 1 minute at 8,000 × *g*.
7. Pipet another 100 µL Wash Buffer 2 (W2) into the RNA purification column, then centrifuge for 2 minutes at 16,000 × *g*. Check the RNA purification column for any residual wash buffer. If wash buffer remains recentrifuge at 16,000 × *g* for 1 minute.

   IMPORTANT! Remove all traces of wash buffer prior to transferring RNA purification column to the new microcentrifuge tube. To remove wash buffer, discard flowthrough waste and recentrifuge the RNA purification column for 1 minute at 16,000 × *g*.
8. Transfer the RNA purification column to a new 0.5 mL microcentrifuge tube provided in the kit.

9. Pipet Elution Buffer (EB) directly onto the membrane of the RNA 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 Elution Buffer into the membrane. To select the correct volume of Elution Buffer, see the following table.

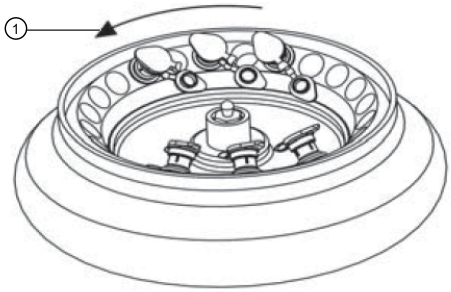
Elution volume guide[1]	
Recommended volume	11 µL
Maximum volume	30 µL

[1] The eluted RNA can be used directly in a 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.

10. Incubate the RNA purification column for 1 minute at room temperature.

11. Centrifuge the RNA purification column for 1 minute at 1,000 × *g* to distribute Elution Buffer in the column, then centrifuge for 1 minute at 16,000 × *g* to elute RNA.

IMPORTANT! To avoid potential breakage of the microcentrifuge tube cap during centrifugation, insert the RNA purification column/0.5 mL tube assembly into a lidless 2 mL tube. 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 ①. *(Optional)* Place an empty, lidless 2 mL tube into the rotor hole adjacent in the clockwise direction to the last assembly (not shown).



① Rotation direction

Note: 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.

The isolated RNA is now ready for use in downstream applications such as reverse transcription or amplification with a RiboAmp™ RNA Amplification Kit.

STOPPING POINT The entire sample may be used immediately or stored at –80°C until use.

Protocol for use with CapSure™ Macro LCM Caps

RNA extraction (CapSure™ Macro LCM Caps)

IMPORTANT! Prior to use, mix Extraction Buffer (XB) thoroughly. Extraction Buffer may form precipitate upon storage. Dissolve precipitate prior to use by mixing thoroughly. If necessary, warm the XB vial at 42°C to redissolve Extraction Buffer prior to use.

1. Dispense Extraction Buffer (XB) and incubate.
   a. Pipet 50 µL Extraction Buffer (XB) into a 0.5 mL microcentrifuge tube (Cat. No. N8010611).
   b. Insert CapSure™ Macro LCM Cap onto the microcentrifuge tube using an LCM Cap Insertion Tool.
   c. Invert the CapSure™ Cap–microcentrifuge tube assembly. Tap the microcentrifuge tube to ensure all Extraction Buffer (XB) is covering the CapSure™ Macro LCM Cap.
   d. Incubate assembly for 30 minutes at 42°C.
2. Centrifuge assembly at 800 × *g* for 2 minutes to collect cell extract into the microcentrifuge tube.
   After centrifugation, the microcentrifuge tube contains the cell extract required to complete the protocol.
3. Remove the CapSure™ Macro LCM Cap and save the microcentrifuge tube with the cell extract in it.

Proceed with RNA isolation protocol (see “RNA isolation (CapSure™ Macro LCM Caps)” on page 14).

STOPPING POINT The cell extract can be stored frozen at –80°C for up to 6 months.

RNA isolation (CapSure™ Macro LCM Caps)

1. Pre-condition the RNA purification column.
   a. Pipet 250 µL Conditioning Buffer (CB) onto the RNA purification column filter membrane.
   b. Incubate the RNA purification column with Conditioning Buffer (CB) for 5 minutes at room temperature.
   c. Centrifuge the RNA purification column in the provided collection tube at 16,000 × *g* for 1 minute.
2. Pipet 50 µL of 70% Ethanol (EtOH) into the cell extract from RNA extraction (see “RNA extraction (CapSure™ Macro LCM Caps)” on page 14), then mix well by pipetting up and down.

   IMPORTANT! Do not vortex.
3. Pipet the cell extract and EtOH mixture into the preconditioned RNA purification column.
   The cell extract and EtOH will have a combined volume of approximately 100 µL.

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

   Note: 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. Ensure that the accumulated flowthrough waste does not make contact with the RNA purification column. Flowthrough waste should be discarded when the waste fluid level approaches the surface of the RNA purification column.

5. Pipet 100 µL Wash Buffer (W1) into the RNA purification column, then centrifuge for 1 minute at 8,000 × *g*.

   (Optional) DNA may be removed by DNase treatment from the preparation at this step. For a detailed protocol, see "DNase treatment" on page 19.

   Note: DNase treatment is recommended if performing reverse transcription or amplification with a RiboAmp™ RNA Amplification Kit after RNA isolation.

6. Pipet 100 µL Wash Buffer 2 (W2) into the RNA purification column, then centrifuge for 1 minute at 8,000 × *g*.

7. Pipet another 100 µL Wash Buffer (W2) into the RNA purification column, then centrifuge for 2 minutes at 16,000 × *g*. Check the RNA purification column for any residual wash buffer. If wash buffer remains recentrifuge at 16,000 × *g* for 1 minute.

   IMPORTANT! Remove all traces of wash buffer prior to transferring RNA purification column to the new microcentrifuge tube. To remove wash buffer, discard flowthrough waste and recentrifuge the RNA purification column for 1 minute at 16,000 × *g*.

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

9. Pipet Elution Buffer (EB) directly onto the membrane of the RNA 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 Elution Buffer into the membrane. To select the correct volume of Elution Buffer, see the following table.

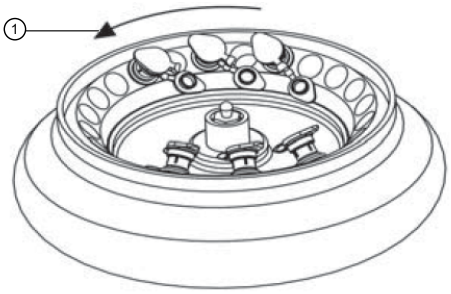
Elution volume guide[1]	
Recommended volume	11 µL
Maximum volume	30 µL

[1] The eluted RNA can be used directly in a 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.

10. Incubate the RNA purification column for 1 minute at room temperature.

11. Centrifuge the RNA purification column for 1 minute at 1,000 × *g* to distribute Elution Buffer in the column, then centrifuge for 1 minute at 16,000 × *g* to elute RNA.

IMPORTANT! To avoid potential breakage of the microcentrifuge tube cap during centrifugation, insert the RNA purification column/0.5 mL tube assembly into a lidless 2 mL tube. 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 ①. *(Optional)* Place an empty, lidless 2 mL tube into the rotor hole adjacent in the clockwise direction to the last assembly (not shown).



① Rotation direction

Note: 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.

The isolated RNA is now ready for use in downstream applications such as reverse transcription or amplification with a RiboAmp™ RNA Amplification Kit.

STOPPING POINT The entire sample may be used immediately or stored at –80°C until use.

A

Troubleshooting

Observation	Possible cause	Recommended action
Isolated RNA is of poor quality	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.	Verify quality of source tissue of LCM cells. Tissue should be embedded and frozen immediately upon removal to reduce RNase activity. For suggestions on verifying quality, call Technical Support.
	Specialized staining protocols and reagents are required for optimal RNA preservation in LCM samples.	Use the HistoGene™ LCM Frozen Section Staining Kit to prepare slides for LCM. The HistoGene™ LCM Frozen Section Staining Kit has been developed and validated for preparing and staining tissues for LCM while maintaining RNA integrity.
	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.	Perform LCM immediately after preparing LCM slides.
	Extraction Buffer (XB) stabilizes RNA by denaturing nucleases.	Pipet Extraction Buffer onto cells acquired by LCM immediately after cell capture. Complete RNA extraction with the PicoPure™ RNA Isolation Kit protocol without stopping. The cell extract resulting from the completion of RNA extraction may be stored in Extraction Buffer at –80°C for up to 6 months.
	Using tissue or cell samples that have been subjected to cross-linking fixative such as formalin or paraformaldehyde diminishes RNA quality.	Use only frozen or alcohol-fixed tissue or cytosmeared cells. 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 Arcturus™ Paradise™ PLUS RNA Extraction and Isolation Kit.
RNA yield is low	RNA integrity has been compromised. Poor quality RNA may not bind effectively to the RNA purification column membrane, decreasing overall RNA yield.	Verify quality of initial tissue sample or LCM slide (see “Isolated RNA is of poor quality”).

Observation	Possible cause	Recommended action
RNA yield is low *(continued)*	Buffer concentrations in extraction mixtures are incorrect due to inadequate mixing with 70% Ethanol (EtOH).	Ensure all buffers are completely mixed and all solids are dissolved prior to use.
	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 on the RNA purification column filter membrane will alter the concentration of Elution Buffer, resulting in poor RNA elution. If any Wash Buffer 2 remains in or on the RNA purification column, recentrifuge it to remove the residual buffer before proceeding to elution.
	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.

B

Supplemental information

DNase treatment

DNase treatment may be performed directly within the RNA 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™, Cat. No. 79254) may be used.

This protocol is applied between step 5 and step 6 in "RNA isolation (CapSure™ HS LCM Caps)" or step 5 and step 6 in "RNA isolation (CapSure™ Macro LCM Caps)".

1. Pipet 5 µL DNase I stock solution into 35 µL Buffer RDD (provided with RNase-Free DNase Set), then mix by gently inverting.

   For isolations from larger samples such as cell pellets (see "RNA extraction from cell pellets"), pipet 10 µL DNase I stock solution into 30 µL Buffer RDD.

2. Pipet the 40 µL DNase incubation mix directly into the RNA purification column membrane, then incubate at room temperature for 15 minutes.

3. Pipet 40 µL PicoPure™ RNA Isolation Kit Wash Buffer 1 (W1) into the RNA purification column membrane, then centrifuge at 8,000 × *g* for 15 seconds.

Proceed with the RNA isolation protocol (see step 6 in "RNA isolation (CapSure™ HS LCM Caps)" or step 6 in "RNA isolation (CapSure™ Macro LCM Caps)").

RNA extraction from cell pellets

The PicoPure™ RNA Isolation Kit may be used for non-LCM samples such as cell sample cultures in suspension. 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 proceeding.

Note: 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 (for example, whole blood samples) directly onto the RNA purification columns is not recommended.

This protocol replaces either RNA extraction protocol (see "RNA extraction (CapSure™ HS LCM Caps)" or "RNA extraction (CapSure™ Macro LCM Caps)").

1. Pellet cells in a RNase-free microcentrifuge tube (**MLS**) by centrifuging at 3,000 × *g* for 10 minutes.

2. Properly dispose of the supernatant.

3. Resuspend the cell pellet in 1 mL of cell suspension media (0.9 mL of 1X PBS/10% BSA; 0.1 mL of 0.5 M EDTA).

   IMPORTANT! Do not vortex.

4. Centrifuge the cell suspension at 3,000 × *g* for 5 minutes.

5. Properly dispose of the supernatant.

6. Extract the cells with 100 µL of Extraction Buffer (XB). Resuspend the cell pellet gently by pipetting.

   IMPORTANT! Do not vortex.

7. Incubate at 42°C for 30 minutes.

8. Centrifuge the sample at 3,000 × *g* for 2 minutes.

9. Pipet the supernatant containing the extracted RNA into a new microcentrifuge tube, avoiding pick-up of pelleted material.

Proceed with RNA isolation or freeze at –80°C.

For RNA isolation, see "RNA isolation (CapSure™ HS LCM Caps)" or "RNA isolation (CapSure™ Macro LCM Caps)" with the following modification to step 2:

Pipet 100 µL of 70% Ethanol (EtOH) to the cell extract (or equal volume to the cell extract), then mix well by pipetting up and down. The combined volume will be approximately 200 µL.

IMPORTANT! Do not vortex.

C

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! **PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES.** Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

Documentation and support

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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