Arcturus™ CapSure™ LCM MicroCaps

USER BULLETIN

Procedures for isolating DNA and RNA from laser capture microdissections

Catalog Numbers A30153, A30154, A30155

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Revision B.0

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

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Revision history: Pub. No. MAN0014607

Revision	Date	Description
B.0	3 February 2017	Product name correction. Minor edits for style or clarification.
A.0	March 2016	New document.

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Contents

1 Overview

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Arcturus™ PicoPure™ DNA Extraction Kit User Guide* (Pub. No. 12637-00), the *Arcturus™ PicoPure™ RNA Isolation Kit User Guide* (Pub. No. 12682-00ARC), and the *Arcturus™ Paradise™ PLUS Reagent System User Guide* (Pub. No. 12872–00). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Arcturus™ Laser Capture Microdissection (LCM) system enables microdissection of single cells or small numbers of cells from fresh-frozen or formalin-fixed, paraffin-embedded (FFPE) tissues.

The Applied Biosystems™ CapSure™ LCM MicroCaps can capture a diameter of up to 4,000 µm and are compatible with both 0.2- and 0.5-mL reaction tubes for nucleic acid isolation after LCM.

Compatibility with both reaction tube sizes allows for:

- Streamlined and complete workflows for a wide variety of downstream applications (go to **thermofisher.com/lcm** for more information)
- Fewer steps where samples are transferred
- Flexibility to use a smaller volume for the initial nucleic acid isolation step
- Reproducible results when working with a small number of cells

A 0.2-mL reaction tube allows amplification of DNA or RNA directly from the tissue lysate (go to **thermofisher.com/lcm** for more information).

Purpose of the User Bulletin

This User Bulletin provides procedures for isolating DNA and RNA from fresh-frozen and FFPE cells using 0.2- and 0.5-mL reaction tubes. For these procedures, see Table 1.

For slide preparation, staining, and cell capture procedures, see the User Guides listed in Table 2.

Table 1 Procedures provided in this User Bulletin

Nucleic acid	Starting tissue type	Reaction tube size	User Bulletin page	Nucleic acid isolation and/or extraction kit	Reference User Guide[1]
DNA	Fresh-frozen or FFPE	0.2 mL	12	PicoPure™ DNA Extraction Kit (Cat. No. KIT0103)	*Arcturus™ PicoPure™ DNA Extraction Kit User Guide* (Pub. No. 12637-00)[2]
		0.5 mL	13		
RNA	Fresh-frozen	0.2 mL	20	PicoPure™ RNA Isolation Kit (Cat. No. KIT0204)	*Arcturus™ PicoPure™ RNA Isolation Kit User Guide* (Pub. No. 12682-00ARC) [2]
		0.5 mL	22		
	FFPE	0.2 mL	29	Arcturus™ Paradise™ PLUS RNA Extraction and Isolation Kit (Cat. No. KIT0312-I)	*Arcturus™ Paradise™ PLUS Reagent System User Guide* (Pub. No. 12872–00)[2]
		0.5 mL	31		

[1] See the indicated User Guide for background information and additional procedures.

[2] The protocol for CapSure™ Macro LCM Caps is superseded by the protocol for a 0.5-mL reaction tube using CapSure™ LCM MicroCaps.

Note: The protocol for CapSure™ HS LCM Caps has not changed. See the *Arcturus™ Paradise™ PLUS Reagent System User Guide*.

Table 2 Procedures for slide preparation, staining, and cell capture

For...	See...
Slide preparation and staining procedures	• *Arcturus™ HistoGene™ Frozen Section Staining Kit User Guide* (Pub. No. 12294–00) for fresh-frozen samples (DNA and RNA) • *Arcturus™ Paradise™ PLUS Reagent System User Guide* (Pub. No. 12872–00) for FFPE samples (DNA and RNA)
Cell capture procedures	*Arcturus^{XT}™ Microdissection Instrument User Guide* (Pub. No. 0112–0153)

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Instruments and software	
Arcturus$^{XT™}$ Microdissection Instrument	Contact your local sales office.
Arcturus$^{XT™}$ Operating Software, v3.4 or later	Go to **thermofisher.com/lcm**, then click **LCM software** under Resources at the bottom of the page.
An Applied Biosystems™ thermal cycler or PCR system that can hold a MicroAmp™ 96-Well Tray (for DNA isolation in 0.2-mL tubes only)	Contact your local sales office.
Equipment	
Incubators: • 65°C for DNA isolation (both 0.2- and 0.5-mL reaction tubes) • 95°C for DNA isolation in 0.5-mL reaction tubes • 42°C for RNA isolation from fresh-frozen samples • 37°C for RNA isolation from FFPE samples	MLS
Microcentrifuge	MLS
MicroCap Incubation Block Base	A30843
MicroCap Incubation Block Top (for 0.2-mL reaction tubes)	A30846
Pipettors	MLS
Consumables	
Disposable gloves	MLS
Pipette tips (nuclease-free)	MLS
Materials for slide preparation and staining	
Materials described in the following User Guides: *Arcturus™ HistoGene™ Frozen Section Staining Kit User Guide* (for fresh-frozen DNA and RNA) *Arcturus™ Paradise™ PLUS Reagent System User Guide* (for FFPE DNA and RNA)	 Pub. No. 12294–00 Pub. No. 12872–00
Materials for cell capture	
Arcturus™ CapSure™ LCM MicroCaps	A30154

Item	Source
One of the following:	
MicroAmp™ Reaction Tube without Cap, 0.2 mL	N8010533
GeneAmp™ Thin-Walled Reaction Tube, with domed cap, 0.5 mL	N8010611
Domed 8-Strip Caps (for MicroAmp™ Reaction Tube without Cap, 0.2 mL only)	Thermo Scientific™ 3418C
Other materials described in the *Arcturus^{XT}™ Microdissection Instrument User Guide*	Pub. No. 0112–0153
Materials for DNA isolation	
PicoPure™ DNA Extraction Kit	KIT0103
MicroAmp™ 96-Well Tray/Retainer Set (for 0.2-mL tubes only)	403081
MicroAmp™ 96-Well Base (for 0.2-mL tubes only)	N8010531
MicroAmp™ Optical Film Compression Pad (for 0.2-mL tubes only)	4312639
Other materials described in the *Arcturus™ PicoPure™ DNA Extraction Kit User Guide*	Pub. No. 12637-00
Materials for RNA isolation from fresh-frozen samples	
PicoPure™ RNA Isolation Kit	KIT0204
(Optional) PureLink™ DNase	12185010
Other materials described in the *Arcturus™ PicoPure™ RNA Isolation Kit User Guide*	Pub. No. 12682-00ARC
Materials for RNA isolation from FFPE samples	
Arcturus™ Paradise™ PLUS RNA Extraction and Isolation Kit (for FFPE samples)	KIT0312-I
Other materials described in the *Arcturus™ Paradise™ PLUS Reagent System User Guide*	Pub. No. 12872–00

2 Isolate DNA from fresh-frozen or FFPE samples

The DNA isolation procedures are compatible with 0.2- and 0.5-mL reaction tubes. See "Required materials not supplied" on page 7 for validated reaction tubes.

This procedure uses materials in the PicoPure™ DNA Extraction Kit. Use this section of the User Bulletin in conjunction with the *Arcturus™ PicoPure™ DNA Extraction Kit User Guide*.

Before you begin

- Before cell capture, prepare the reaction tubes with Proteinase K:
  a. Add 155 µL of Reconstitution Buffer to one vial of Proteinase K.
  b. Vortex gently to dissolve the Proteinase K, then place on ice.

  IMPORTANT! Excessive mixing can denature Proteinase K. Use the prepared Proteinase K within 12 hours.

  c. Add the prepared Proteinase K to each reaction tube:

Reaction tube	Volume per tube
0.2-mL	10–50 µL[1]
0.5-mL	50 µL

[1] For efficient lysis, use a sufficient volume of prepared Proteinase K to ensure that the film on the CapSure™ LCM MicroCaps is fully covered by Proteinase K.

- Preheat the incubator to 65°C.
- *(0.2-mL tubes only)* Preheat the MicroCap Incubation Block Top to 65°C.

Capture cells and assemble the prepared reaction tubes with CapSure™ LCM MicroCaps

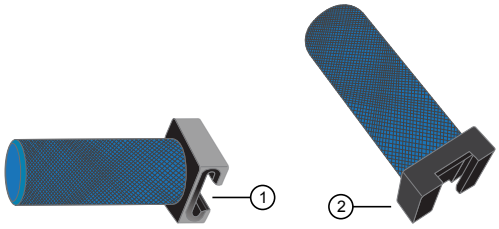


Figure 1 LCM Cap Insertion Tool

① Open side to slide over the LCM MicroCap ② Closed side

1. After capturing cells, move the LCM MicroCap to the QC Caps area of the modular stage insert.

   See the *Arcturus$^{XT™}$ Microdissection Instrument User Guide* for cell-capture procedures.

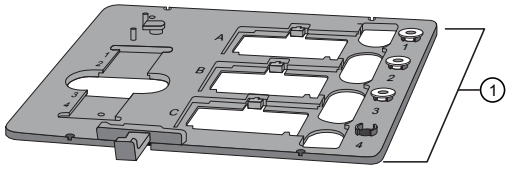
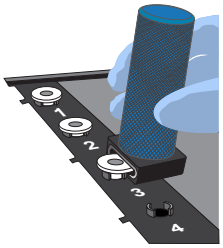


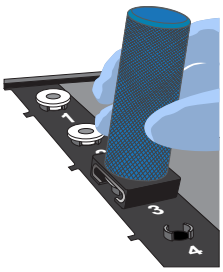
Figure 2 Arcturus$^{XT™}$ Microdissection Instrument modular stage insert

① QC Caps area

2. Align the opening of the insertion tool with the LCM MicroCap in the QC Caps area.

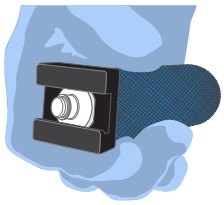


3. Slide the insertion tool over the LCM MicroCap until it is fully engaged.



4. Pull the insertion tool–LCM MicroCap assembly out of the QC Station.

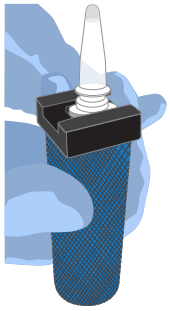
   The LCM MicroCap should be completely inside the insertion tool.



5. Insert a reaction tube prepared with Proteinase K (page 9) onto each LCM MicroCap, then press firmly on the insertion tool to ensure a tight and even seal.

6. Invert the insertion tool with the LCM MicroCap–reaction tube assembly, then shake gently so that Proteinase K completely covers the LCM MicroCap surface.

   If needed, tap the assembly to remove air bubbles. For efficient lysis, the Proteinase K must completely cover the captured cells.



7. Remove the insertion tool from the LCM MicroCap–reaction tube assembly, keeping the assembly inverted.

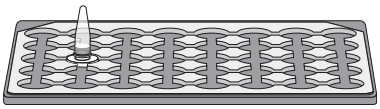
   Set the assembly aside in an inverted position.

8. Repeat steps 2 through 7 for each LCM MicroCap remaining in the QC Caps area.

Proceed immediately to “Prepare the cell lysate (0.2-mL tubes)“ on page 12 or “Prepare the cell lysate (0.5-mL tubes)“ on page 13, depending on the size of reaction tube used.

Prepare the cell lysate (0.2-mL tubes)

1. Place the inverted LCM MicroCap–reaction tube assembly on the MicroCap Incubation Block Base, ensuring that the reaction tube is perpendicular to the top of the block base.



2. Cover the LCM MicroCap–reaction tube assembly and block base with the preheated MicroCap Incubation Block Top.

3. Incubate at 65°C in the incubator:

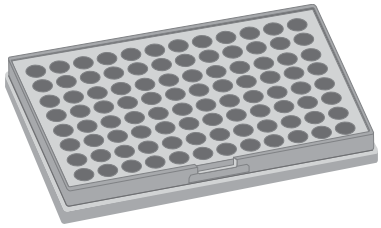
Sample type	Incubation time
Fresh-frozen	3 hours[1]
FFPE	16 hours[1]

[1] Optimize the incubation times; some tissue types may require a longer incubation.

4. Centrifuge the LCM MicroCap–reaction tube assembly at 1,000 × *g* for 1 minute.

5. Remove the LCM MicroCap.
   The cell lysate is in the reaction tube.

6. Place each reaction tube in a MicroAmp™ 96-Well Tray/Retainer Set, cap the reaction tubes, then load into the thermal cycler:
   a. Place the MicroAmp™ 96-Well Tray on the MicroAmp™ 96-Well Base.

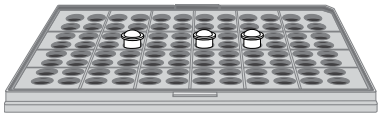


   b. Place each reaction tube onto the tray, then place the retainer over the reaction tubes.

   c. Cap each reaction tube with a cap from the Domed 8-Strip Caps.

d. Lift the tray-retainer assembly containing the capped reaction tubes from the base, then load it onto the thermal cycler block.

   The well of the retainer labeled A1 should be located in the upper left corner.



e. Cover the tray-retainer assembly containing the capped reaction tubes with a MicroAmp™ Optical Film Compression Pad.

f. Close the thermal cycler.

7. Incubate at 95°C for 10 minutes to inactivate the Proteinase K, then cool to room temperature.

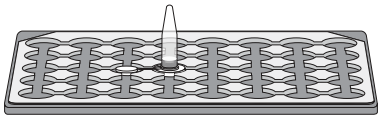
8. Replace each cap with a new cap from the Domed 8-Strip Caps.

Store the samples at –80°C or proceed to:

- Preamplification (see the *TaqMan® PreAmp Master Mix Kit Protocol* (Pub. No. 4384557)
- PCR analysis, with or without preamplification
- Other downstream workflows (see "Products for downstream applications" on page 33)

Prepare the cell lysate (0.5-mL tubes)

1. Place the inverted LCM MicroCap–reaction tube assembly on the MicroCap Incubation Block Base, ensuring that the reaction tube is perpendicular to the top of the block base.



2. Incubate the LCM MicroCap-reaction tube assembly at 65°C in the incubator:

Sample type	Incubation time
Fresh-frozen	≥3 hours
FFPE	≥16 hours

3. Centrifuge the LCM MicroCap–reaction tube assembly at 1,000 × *g* for 1 minute.

4. Remove the LCM MicroCap, then cap the reaction tube.

   The cell lysate is in the reaction tube.

5. Incubate at 95°C for 10 minutes in the incubator to inactivate the Proteinase K, then cool to room temperature.

Store the samples at –80°C or proceed to:

- Preamplification (see the *TaqMan® PreAmp Master Mix Kit Protocol* (Pub. No. 4384557)
- PCR analysis, with or without preamplification
- Other downstream workflows (see "Products for downstream applications" on page 33)

3 Isolate and purify RNA from fresh-frozen samples

The RNA isolation and purification procedures are compatible with 0.2- and 0.5-mL reaction tubes. See "Required materials not supplied" on page 7 for validated reaction tubes.

This procedure uses materials in the PicoPure™ RNA Isolation Kit. Use this section of the User Bulletin in conjunction with the *Arcturus™ PicoPure™ RNA Isolation Kit User Guide*.

Before you begin (preparing the cell lysate)

- Before cell capture, prepare the reaction tubes with Extraction Buffer:
  a. If a precipitate has formed, mix the Extraction Buffer thoroughly to ensure that the precipitate is dissolved.

     If needed, warm to dissolve the precipitate.

  b. Add the prepared Extraction Buffer to each reaction tube:

Reaction tube	Volume per tube
0.2-mL	10–50 µL[1]
0.5-mL	50 µL

[1] For efficient lysis, use a sufficient volume of prepared Extraction Buffer to ensure that the film on the CapSure™ LCM MicroCaps is fully covered by Extraction Buffer.

- Preheat the incubator to 42°C.
- *(0.2-mL tubes only)* Preheat the MicroCap Incubation Block Top to 42°C.

Capture cells and assemble the prepared reaction tubes with CapSure™ LCM MicroCaps

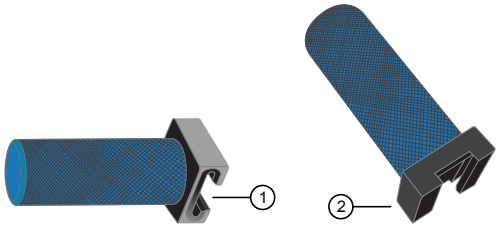


Figure 3 LCM Cap Insertion Tool

① Open side to slide over the LCM MicroCap ② Closed side

1. After capturing cells, move the LCM MicroCap to the QC Caps area of the modular stage insert.

   See the *Arcturus$^{XT™}$ Microdissection Instrument User Guide* for cell-capture procedures.

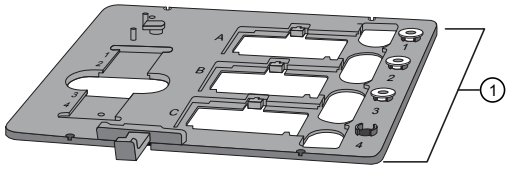
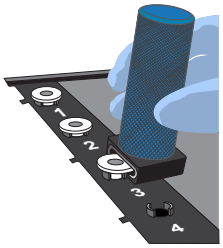


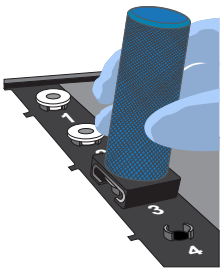
Figure 4 Arcturus$^{XT™}$ Microdissection Instrument modular stage insert

① QC Caps area

2. Align the opening of the insertion tool with the LCM MicroCap in the QC Caps area.

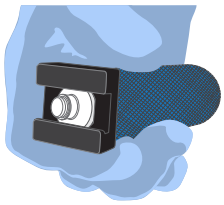


3. Slide the insertion tool over the LCM MicroCap until it is fully engaged.



4. Pull the insertion tool–LCM MicroCap assembly out of the QC Station.

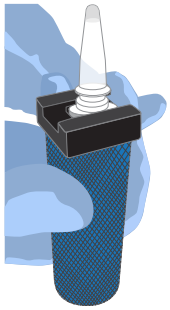
   The LCM MicroCap should be completely inside the insertion tool.



5. Insert a reaction tube prepared with Extraction Buffer (page 15) onto each LCM MicroCap, then press firmly on the insertion tool to ensure a tight and even seal.

6. Invert the insertion tool with the LCM MicroCap–reaction tube assembly, then shake gently so that Extraction Buffer completely covers the LCM MicroCap surface.

   If needed, tap the assembly to remove air bubbles. For efficient lysis, the Extraction Buffer must completely cover the captured cells.



7. Remove the insertion tool from the LCM MicroCap–reaction tube assembly, keeping the assembly inverted.

   Set the assembly aside in an inverted position.

8. Repeat steps 2 through 7 for each LCM MicroCap remaining in the QC Caps area.

Proceed immediately to “Prepare the cell lysate from fresh-frozen samples“ on page 18.

Prepare the cell lysate from fresh-frozen samples

This procedure applies to both 0.2- and 0.5-mL reaction tubes.

1. Place the LCM MicroCap–reaction tube assembly on the MicroCap Incubation Block Base, ensuring that the reaction tube is perpendicular to the top of the block base.

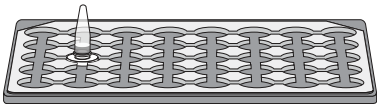


Figure 5 MicroCap Incubation Block Base with 0.2-mL reaction tube

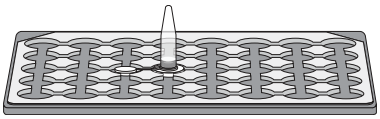
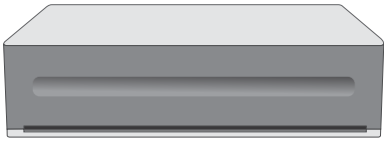


Figure 6 MicroCap Incubation Block Base with 0.5-mL reaction tube

2. *(0.2-mL tubes only)* Cover the LCM MicroCap–reaction tube assembly and block base with a preheated MicroCap Incubation Block Top.



3. Incubate at 42°C for 30 minutes.
4. Centrifuge the LCM MicroCap–reaction tube assembly at 800 × *g* for 2 minutes.
5. Remove the LCM MicroCap.
   The cell lysate is in the reaction tube.

Proceed to "Before you begin (purifying the RNA)" on page 20.

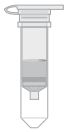
Alternatively, cap the reaction tubes and store at –80°C. Use the following caps:

- **0.2-mL reaction tube**—Use a cap from the Domed 8-Strip Caps.
- **0.5-mL reaction tube**—Use the domed cap provided with the tube.

General workflow for RNA purification

Precondition the column

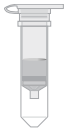
(pages 20 and 22)



Conditioning Buffer, room temperature

Transfer the lysate to the column and bind RNA

(pages 20 and 22)



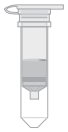
To column: 70% ethanol + cell lysate

x 2

Wash Buffer 1

Treat with DNase and wash the RNA

(pages 21 and 22)



Optional: DNase, room temperature

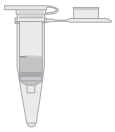
Wash Buffer 1

Wash Buffer 2

x 2

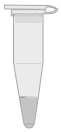
Elute the RNA

(pages 21 and 23)



Elution Buffer, room temperature

x 2



RNA

: centrifugation

Before you begin (purifying the RNA)

- *(If the cell lysate was frozen)* Thaw the cell lysate within the reaction tube on ice or at room temperature, then centrifuge at 800 × *g* for 2 minutes.

  IMPORTANT! Do not leave the reaction tube at room temperature for longer than 15 minutes.

- *(Optional)* Prepare PureLink™ DNase.

  PureLink™ DNase is not included in the PicoPure™ RNA Isolation Kit. See "Required materials not supplied" on page 7. 10X DNase I Reaction Buffer and RNase-Free Water are included with PureLink™ DNase.

  a. Add 550 µL of RNase-Free Water to the vial of lyophilized PureLink™ DNase.

  b. Dilute the 10X DNase I Reaction Buffer to a 2X solution using RNase-Free Water.

  c. Combine 10 µL of reconstituted PureLink™ DNase with 40 µL of 2X DNase I Reaction Buffer for each sample, plus 10% overage.

  d. Invert to mix, then centrifuge briefly.

  Note: Do not vortex.

Purify the RNA from fresh-frozen samples (0.2-mL tube)

Precondition the RNA Purification Column

1. Pipet 250 µL of Conditioning Buffer onto the RNA Purification Column filter membrane.
2. Incubate for 5 minutes at room temperature.
3. Centrifuge the column in a collection tube at 16,000 × *g* for 1 minute, then discard the liquid in the collection tube.

Transfer the lysate to the column and bind RNA

1. Add 10 µL of 70% ethanol to the cell lysate in the reaction tube, mix by pipetting, then transfer the full volume to a preconditioned column.

   IMPORTANT! Do not centrifuge the cell lysate after adding 70% ethanol.

2. Centrifuge at 100 × *g* for 2 minutes to bind the RNA, centrifuge again at 16,000 × *g* for 30 seconds to remove the flowthrough, then discard the liquid in the collection tube.
3. Add 100 µL of Wash Buffer 1 to the column, centrifuge at 8,000 × *g* for 1 minute, then discard the liquid in the collection tube.

Treat with DNase and wash the RNA

1. *(Optional)* Add 50 µL of DNase (page 20) to the column, then incubate at room temperature for 20 minutes.

   We recommend treating with DNase for the following downstream applications:

   - Reverse transcription
   - RNA amplification
   - Preamplification
   - qPCR

2. Add 40 µL of Wash Buffer 1 to the column, centrifuge at 8,000 × *g* for 15 seconds, then discard the liquid in the collection tube.

3. Add 100 µL of Wash Buffer 2 to the column, centrifuge at 8,000 × *g* for 1 minute, then discard the liquid in the collection tube.

4. Add 100 µL of Wash Buffer 2 to the column, then centrifuge at 16,000 × *g* for 2 minutes.

   If there is residual wash buffer in the column, centrifuge again at 16,000 × *g* for 1 minute.

Elute the RNA

1. Transfer the column to a 0.5-mL microcentrifuge tube.

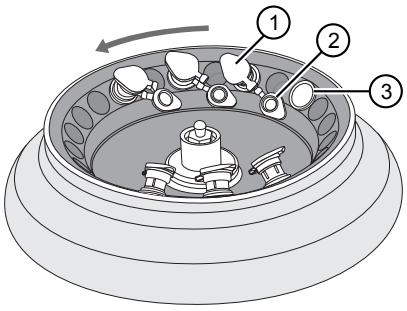
2. Add 14 µL of Elution Buffer directly onto the column membrane by gently touching the membrane surface with the pipette tip.

   We recommend 14 µL of Elution Buffer, but you can use up to 30 µL. You can concentrate the eluted RNA in a vacuum centrifuge to reduce the volume to 10 µL.

3. Incubate at room temperature for 1 minute.

4. Centrifuge at 1,000 × *g* for 1 minute to distribute the Elution Buffer within the column, then centrifuge again at 16,000 × *g* for 1 minute to elute the RNA.

   To prevent the microcentrifuge caps from breaking during centrifugation: Place lidless 2-mL tubes into the centrifuge, then place each column-microcentrifuge tube assembly inside a lidless 2-mL tube. Position the collection tubes so that the caps trail the tubes. Place one extra lidless 2-mL tube adjacent to the last assembly in the clockwise direction.



① Column-microcentrifuge tube assembly inside a lidless 2-mL tube
② Collection tube cap, trailing the tube
③ One extra lidless 2-mL tube

Proceed to the application of interest ("Products for downstream applications" on page 33) or store at –80°C.

Purify the RNA from fresh-frozen samples (0.5-mL tube)

Precondition the RNA Purification Column

1. Pipet 250 µL of Conditioning Buffer onto the RNA Purification Column filter membrane.
2. Incubate for 5 minutes at room temperature.
3. Centrifuge the column in a collection tube at 16,000 × *g* for 1 minute, then discard the liquid in the collection tube.

Transfer the lysate to the column and bind RNA

1. Add 50 µL of 70% ethanol to the cell lysate in the reaction tube, mix by pipetting, then transfer the full volume to a preconditioned column.

   IMPORTANT! Do not centrifuge the cell lysate after adding 70% ethanol.

2. Centrifuge at 100 × *g* for 2 minutes to bind the RNA, centrifuge again at 16,000 × *g* for 30 seconds to remove the flowthrough, then discard the liquid in the collection tube.
3. Add 100 µL of Wash Buffer 1 to the column, centrifuge at 8,000 × *g* for 1 minute, then discard the liquid in the collection tube.

Treat with DNase and wash the RNA

1. *(Optional)* Add 50 µL of DNase (page 20) to the column, then incubate at room temperature for 20 minutes.

   We recommend treating with DNase for the following downstream applications:

   - Reverse transcription
   - RNA amplification
   - Preamplification
   - qPCR

2. Add 40 µL of Wash Buffer 1 to the column, centrifuge at 8,000 × *g* for 15 seconds, then discard the liquid in the collection tube.
3. Add 100 µL of Wash Buffer 2 to the column, centrifuge at 8,000 × *g* for 1 minute, then discard the liquid in the collection tube.
4. Add 100 µL of Wash Buffer 2 to the column, then centrifuge at 16,000 × *g* for 2 minutes.

   If there is residual wash buffer in the column, centrifuge again at 16,000 × *g* for 1 minute.

Elute the RNA

1. Transfer the column to a 0.5-mL microcentrifuge tube.

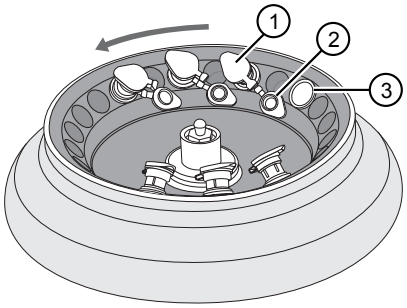
2. Add 14 µL of Elution Buffer directly onto the column membrane by gently touching the membrane surface with the pipette tip.

   We recommend 14 µL of Elution Buffer, but you can use up to 30 µL. You can concentrate the eluted RNA in a vacuum centrifuge to reduce the volume to 10 µL.

3. Incubate at room temperature for 1 minute.

4. Centrifuge at 1,000 × *g* for 1 minute to distribute the Elution Buffer within the column, then centrifuge again at 16,000 × *g* for 1 minute to elute the RNA.

   To prevent the microcentrifuge caps from breaking during centrifugation: Place lidless 2-mL tubes into the centrifuge, then place each column-microcentrifuge tube assembly inside a lidless 2-mL tube. Position the collection tubes so that the caps trail the tubes. Place one extra lidless 2-mL tube adjacent to the last assembly in the clockwise direction.



① Column-microcentrifuge tube assembly inside a lidless 2-mL tube
② Collection tube cap, trailing the tube
③ One extra lidless 2-mL tube

Proceed to the application of interest ("Products for downstream applications" on page 33) or store at –80°C.

4 Isolate and purify RNA from FFPE samples

The RNA isolation and purification procedures are compatible with 0.2- and 0.5-mL reaction tubes. See "Required materials not supplied" on page 7 for validated reaction tubes.

This procedure uses materials in the Arcturus™ Paradise™ PLUS RNA Extraction and Isolation Kit. Use this section of the User Bulletin in conjunction with the *Arcturus™ Paradise™ PLUS Reagent System User Guide*.

Before you begin (preparing the cell lysate)

- Before cell capture, prepare the reaction tubes with Proteinase K:
  a. Add 300 µL of Reconstitution Buffer to one vial of Pro K Mix (600 µg).
  b. Vortex gently to dissolve the Pro K Mix, then place on ice.
     The resulting mixture is Proteinase K.

     IMPORTANT! Excessive mixing can denature Proteinase K. Use the mixed Proteinase K within 12 hours.

  c. Add the prepared Proteinase K to each reaction tube:

Reaction tube	Volume per tube
0.2-mL	10–50 µL[1]
0.5-mL	50 µL

[1] For efficient lysis, use a sufficient volume of prepared Proteinase K to ensure that the film on the CapSure™ LCM MicroCaps is fully covered by Proteinase K.

- Preheat the incubator to 37°C.
- *(0.2-mL tubes only)* Preheat the MicroCap Incubation Block Top to 37°C.

Capture cells and assemble the prepared reaction tubes with CapSure™ LCM MicroCaps

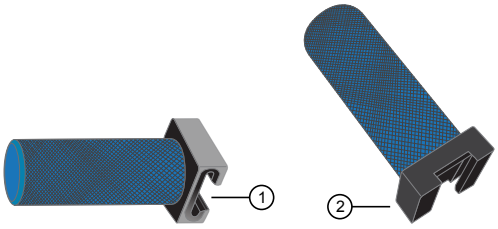


Figure 7 LCM Cap Insertion Tool

① Open side to slide over the LCM MicroCap ② Closed side

1. After capturing cells, move the LCM MicroCap to the QC Caps area of the modular stage insert.

   See the *Arcturus$^{XT™}$ Microdissection Instrument User Guide* for cell-capture procedures.

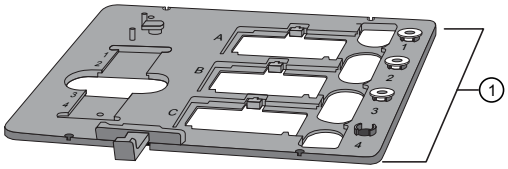
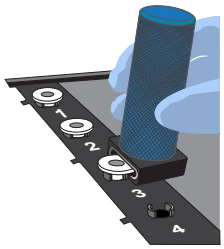


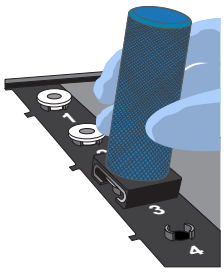
Figure 8 Arcturus$^{XT™}$ Microdissection Instrument modular stage insert

① QC Caps area

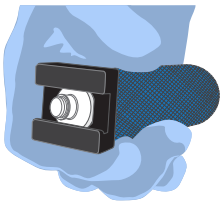
2. Align the opening of the insertion tool with the LCM MicroCap in the QC Caps area.



3. Slide the insertion tool over the LCM MicroCap until it is fully engaged.

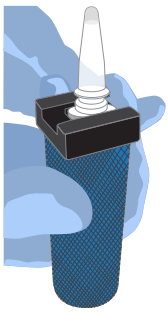


4. Pull the insertion tool–LCM MicroCap assembly out of the QC Station.
   The LCM MicroCap should be completely inside the insertion tool.



5. Insert a reaction tube prepared with Proteinase K (page 24) onto each LCM MicroCap, then press firmly on the insertion tool to ensure a tight and even seal.

6. Invert the insertion tool with the LCM MicroCap–reaction tube assembly, then shake gently so that Proteinase K completely covers the LCM MicroCap surface.
   If needed, tap the assembly to remove air bubbles. For efficient lysis, the Proteinase K must completely cover the captured cells.



7. Remove the insertion tool from the LCM MicroCap–reaction tube assembly, keeping the assembly inverted.
   Set the assembly aside in an inverted position.

8. Repeat steps 2 through 7 for each LCM MicroCap remaining in the QC Caps area.

Proceed immediately to "Prepare the cell lysate from FFPE samples" on page 27.

Prepare the cell lysate from FFPE samples

This procedure applies to both 0.2- and 0.5-mL reaction tubes.

1. Place the LCM MicroCap–reaction tube assembly on the MicroCap Incubation Block Base, ensuring that the reaction tube is perpendicular to the top of the block base.

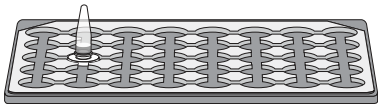


Figure 9 MicroCap Incubation Block Base with 0.2-mL reaction tube

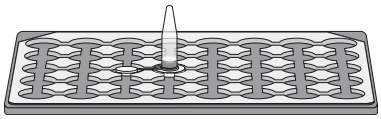
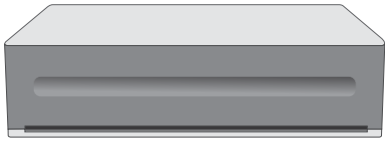


Figure 10 MicroCap Incubation Block Base with 0.5-mL reaction tube

2. *(0.2-mL tubes only)* Cover the LCM MicroCap–reaction tube assembly and block base with a preheated MicroCap Incubation Block Top.



3. Incubate at 37°C for 16 hours.
4. Centrifuge the LCM MicroCap–reaction tube assembly at 800 × *g* for 1 minute.
5. Remove the LCM MicroCap.
   The cell lysate is in the reaction tube.

Proceed to "Before you begin (purifying the RNA)" on page 29.

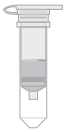
Alternatively, cap the reaction tubes and store at –80°C. Use the following caps:

- **0.2-mL reaction tube**—Use a cap from the Domed 8-Strip Caps.
- **0.5-mL reaction tube**—Use the domed cap provided with the tube.

General workflow for RNA purification

Precondition the column

(pages 29 and 31)



Conditioning Buffer, room temperature

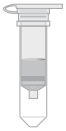
▼

⇄

▼

Transfer the lysate to the column and bind RNA

(pages 29 and 31)



Binding Buffer + cell lysate

▼

To column: Ethanol Solution + Binding Buffer + cell lysate

▼

⇄ x 2

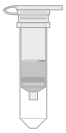
▼

Wash Buffer 1 ⇄

▼

Treat with DNase and wash the RNA

(pages 30 and 31)



DNase, room temperature

▼

Wash Buffer 1 ⇄

▼

Wash Buffer 2 ⇄ x 2

▼

Elute the RNA

(pages 30 and 31)

Elution Buffer, room temperature

▼

⇄ x 2

▼

RNA

⇄ : centrifugation

Before you begin (purifying the RNA)

- *(If the cell lysate was frozen)* Thaw the cell lysate within the reaction tube on ice or at room temperature, then centrifuge at 800 × *g* for 2 minutes.

  IMPORTANT! Do not leave the reaction tube at room temperature for longer than 15 minutes.

- If the Binding Buffer formed a precipitate during storage that does not dissolve by mixing, pre-warm the Binding Buffer to 37°C.

- Prepare the DNase by mixing 2 µL of DNase with 18 µL of DNase Buffer for each sample, plus 10% overage.

  DNase and DNase Buffer are included in the Arcturus™ Paradise™ PLUS RNA Extraction and Isolation Kit.

Purify the RNA from FFPE samples (0.2-mL tube)

Precondition the MiraCol™ Purification Column

1. Pipet 200 µL of Conditioning Buffer onto the MiraCol™ Purification Column filter membrane.
2. Incubate for 5 minutes at room temperature.
3. Centrifuge the column in a collection tube at 16,000 × *g* for 1 minute, then discard the liquid in the collection tube.

Transfer the lysate to the column and bind RNA

1. Mix the Binding Buffer, add 11 µL to the cell lysate in the reaction tube, then pipet to mix.

   IMPORTANT! Do not centrifuge the cell extract after adding the Binding Buffer.

2. Add 21 µL of Ethanol Solution, mix, then transfer the full volume to a preconditioned column.
3. Centrifuge at 100 × *g* for 2 minutes to bind the RNA, centrifuge again at 16,000 × *g* for 1 minute to remove the flowthrough, then discard the liquid in the collection tube.
4. Add 100 µL of Wash Buffer 1 to the column, centrifuge at 8,000 × *g* for 1 minute, then discard the liquid in the collection tube.

Treat with DNase and wash the RNA

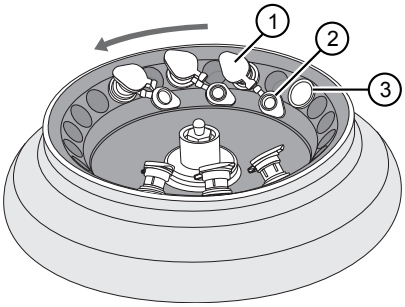
1. Add 20 µL of DNase (page 29) to the column, then incubate at room temperature for 20 minutes.
2. Add 40 µL of Wash Buffer 1 to the column, centrifuge at 8,000 × *g* for 1 minute, then discard the liquid in the collection tube.
3. Add 100 µL of Wash Buffer 2 to the column, centrifuge at 8,000 × *g* for 1 minute, then discard the liquid in the collection tube.
4. Add 100 µL of Wash Buffer 2 to the column, centrifuge at 16,000 × *g* for 2 minutes.

   If there is residual wash buffer in the column, centrifuge again at 16,000 × *g* for 1 minute.

Elute the RNA

1. Transfer the column to a 0.5-mL microcentrifuge tube.
2. Add 14 µL of Elution Buffer directly onto the column membrane by gently touching the membrane surface with the pipette tip.
3. Incubate at room temperature for 1 minute.
4. Centrifuge at 1,000 × *g* for 1 minute to distribute the Elution Buffer within the column, then centrifuge again at 16,000 × *g* for 1 minute to elute the RNA.

   To prevent the microcentrifuge caps from breaking during centrifugation: Place lidless 2-mL tubes into the centrifuge, then place each column-microcentrifuge tube assembly inside a lidless 2-mL tube. Position the collection tubes so that the caps trail the tubes. Place one extra lidless 2-mL tube adjacent to the last assembly in the clockwise direction.



① Column-microcentrifuge tube assembly inside a lidless 2-mL tube
② Collection tube cap, trailing the tube
③ One extra lidless 2-mL tube

Proceed to the application of interest (“Products for downstream applications“ on page 33) or store at –80°C.

Purify the RNA from FFPE samples (0.5-mL tube)

Precondition the MiraCol™ Purification Column

1. Pipet 200 µL of Conditioning Buffer onto the MiraCol™ Purification Column filter membrane.
2. Incubate for 5 minutes at room temperature.
3. Centrifuge the column in a collection tube at 16,000 × *g* for 1 minute, then discard the liquid in the collection tube.

Transfer the lysate to the column and bind RNA

1. Mix the Binding Buffer, add 53 µL to the cell lysate in the reaction tube, then pipet to mix.

   IMPORTANT! Do not centrifuge the cell extract after adding the Binding Buffer.

2. Add 103 µL of Ethanol Solution, mix, then transfer the full volume to a preconditioned column.
3. Centrifuge at 100 × *g* for 2 minutes to bind the RNA, centrifuge again at 16,000 × *g* for 1 minute to remove the flowthrough, then discard the liquid in the collection tube.
4. Add 100 µL of Wash Buffer 1 to the column, centrifuge at 8,000 × *g* for 1 minute, then discard the liquid in the collection tube.

Treat with DNase and wash the RNA

1. Add 20 µL of DNase (page 29) to the column, then incubate at room temperature for 20 minutes.
2. Add 40 µL of Wash Buffer 1 to the column, centrifuge at 8,000 × *g* for 1 minute, then discard the liquid in the collection tube.
3. Add 100 µL of Wash Buffer 2 to the column, centrifuge at 8,000 × *g* for 1 minute, then discard the liquid in the collection tube.
4. Add 100 µL of Wash Buffer 2 to the column, centrifuge at 16,000 × *g* for 2 minutes.

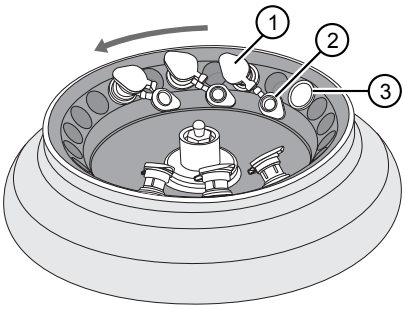
   If there is residual wash buffer in the column, centrifuge again at 16,000 × *g* for 1 minute.

Elute the RNA

1. Transfer the column to a 0.5-mL microcentrifuge tube.
2. Add 14 µL of Elution Buffer directly onto the column membrane by gently touching the membrane surface with the pipette tip.
3. Incubate at room temperature for 1 minute.

4. Centrifuge at 1,000 × *g* for 1 minute to distribute the Elution Buffer within the column, then centrifuge again at 16,000 × *g* for 1 minute to elute the RNA.

   To prevent the microcentrifuge caps from breaking during centrifugation: Place lidless 2-mL tubes into the centrifuge, then place each column-microcentrifuge tube assembly inside a lidless 2-mL tube. Position the collection tubes so that the caps trail the tubes. Place one extra lidless 2-mL tube adjacent to the last assembly in the clockwise direction.



① Column-microcentrifuge tube assembly inside a lidless 2-mL tube
② Collection tube cap, trailing the tube
③ One extra lidless 2-mL tube

Proceed to the application of interest ("Products for downstream applications" on page 33) or store at –80°C.

5 Downstream applications

Products for downstream applications

For this nucleic acid...	And these applications...	Use these products...
DNA from fresh-frozen or FFPE samples	qPCR	• TaqMan® PreAmp Master Mix Kit • TaqMan® Fast Advanced Master Mix
	End-point PCR	• Gel electrophoresis
	Next-generation sequencing (NGS)	• Ion AmpliSeq™ DNA Library Kits
	PCR amplification and CE sequencing	• BigDye™ Direct Cycle Sequencing Kit
RNA from fresh-frozen samples	RT, preamplification, and qPCR	• Arcturus™ RiboAmp™ HS PLUS cDNA Kit • SuperScript™ VILO™ cDNA Synthesis Kit • TaqMan® PreAmp Master Mix Kit • SuperScript™ IV Reverse Transcriptase
	aRNA amplification and microarray	• Arcturus™ RiboAmp™ HS PLUS Kit • Arcturus™ Turbo Labeling™ Kit, biotin • Arcturus™ Turbo Labeling™ Kit, Cy®3 dye • Arcturus™ Turbo Labeling™ Kit, Cy®5 dye
	RT and NGS	• SuperScript™ VILO™ cDNA Synthesis Kit • Ion AmpliSeq™ RNA Library workflow
RNA from FFPE samples	WT-RT and qPCR	• Arcturus™ Paradise™ PLUS Whole Transcript Reverse Transcription Kit • SuperScript™ VILO™ cDNA Synthesis Kit • TaqMan® PreAmp Master Mix Kit
	aRNA amplification and microarray	• Arcturus™ Paradise™ PLUS 2 Round Kit • Arcturus™ Paradise™ PLUS 2 Round Kit, amino allyl • Arcturus™ Paradise™ PLUS 1.5 Round Kit • Arcturus™ Turbo Labeling™ Kit, biotin • Arcturus™ Turbo Labeling™ Kit, Cy®3 dye • Arcturus™ Turbo Labeling™ Kit, Cy®5 dye
	RT and NGS	• SuperScript™ VILO™ cDNA Synthesis Kit • Ion AmpliSeq™ RNA Library workflow

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

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