

# Arcturus® HistoGene® Frozen Section Staining Kit

## User Guide



**For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.**

Information in this document is subject to change without notice.

APPLIED BIOSYSTEMS DISCLAIMS ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. TO THE FULLEST EXTENT ALLOWED BY LAW, IN NO EVENT SHALL APPLIED BIOSYSTEMS BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF, WHETHER OR NOT FORESEEABLE AND WHETHER OR NOT APPLIED BIOSYSTEMS IS ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

**TRADEMARKS**

The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners. Tissue-Tek is a trademark of Sakura FineTek. RNase AWAY is a trademark of Molecular Bio-Products Inc. Kimwipes is a trademark of Kimberly-Clark Corporation. Sensiscript and Qiagen are trademarks of Qiagen Group. Ready-to-Go is a trademark of GE Healthcare Bio-Sciences.

© 2010 Life Technologies Corporation. All rights reserved.

12294-00 Rev. C

07/2010

---

## **I. Introduction**

A. Background	1
B. Storage and Stability	1
C. Safety Data Sheets	2
D. Related Products	2

## **II. Kit Components**

A. Reagents and Supplies in Kit	4
---------------------------------	---

## **III. Preliminary Steps**

A. Material and Protocol Review	5
B. Recommendations for RNase-free Technique	5
C. Additional Lab Equipment and Materials Required	6

## **IV. Protocol**

A. Specimen Freezing	7
B. Slide Preparation	7
C. Staining and Dehydration	9

## **V. Troubleshooting**

A. Targeted Cells do not Lift from the Slide During LCM	11
B. RNA Cannot be Recovered from the Sample	11

## **VI. Appendix**

A. Related Protocols	12
1. Cleaning Plastic Slide Jars	12
2. RNA Extraction and Isolation	12
3. Checking RNA integrity with RT-PCR and Gel Analysis of mRNA from LCM Samples	12
4. Tissue Scrape Protocol for Verifying RNA Quality Using the PicoPure RNA Isolation Kit	14

---

---

# I. Introduction

---

## A. Background

A principal application of LCM (Laser Capture Microdissection) is the analysis of gene expression patterns in cells captured from frozen specimens. Obtaining accurate results from gene expression analysis experiments, including microarray hybridization and quantitative PCR, depends on careful preservation of intact RNA molecules in captured cells.

The Arcturus® HistoGene® LCM Frozen Section Staining Kit is LCM Certified for preparing and staining tissues while preserving intact nucleic acid and protein species from captured cell populations. The kit works with the Arcturus® PicoPure® Extraction Kits and the Arcturus® RiboAmp® PLUS RNA Amplification Kits to provide a complete solution for studying DNA and RNA from cells isolated by LCM.

Research shows that this staining kit enables extraction of high quality RNA from a variety of tissues, including human foreskin, ileum and jejunum, and mouse kidney, liver, brain, salivary gland, thymus and small intestine.

For more information, call Technical Support at 1-800-831-6844 option 5.

---

## B. Storage and Stability

Inspect all kit components upon receipt. Ethanol and Xylene are flammable and should be unpacked and stored at room temperature in a fireproof storage cabinet or fume hood with adequate ventilation. Cap bottles tightly between uses. Store remaining kit supplies at room temperature in a clean, dust-free environment.

---

---

## C. Safety Data Sheets

Safety Data Sheets (SDS) for kit chemical components are available from Technical Support. Call 1-800-831-6844 option 5.

You can also obtain these sheets from:  
[www.appliedbiosystems.com](http://www.appliedbiosystems.com).

---

## D. Related Products from Arcturus

### **PicoPure® RNA Isolation Kit**

For extraction and isolation of total RNA from small samples particularly Laser Capture Microdissected (LCM) cells. The PicoPure RNA Kit comes with optimized buffers, MiraCol™ Purification Columns and an easy-to-use protocol to maximize recovery of high-quality total cellular RNA ready for amplification with the RiboAmp PLUS RNA Amplification Kit.

### **RiboAmp® PLUS RNA Amplification Kit**

The RiboAmp PLUS 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 PLUS Kit comes with all necessary enzymes, reagents, and MiraCol purification columns needed to complete the included amplification protocol.

### **RiboAmp® HS PLUS RNA Amplification Kit**

The RiboAmp HS PLUS 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 for labeling and hybridizing onto expression microarrays. The RiboAmp HS PLUS Kit comes with all necessary enzymes, reagents, and MiraCol Purification Columns needed to complete the included amplification protocol.

**PicoPure® DNA Extraction Kit**

The PicoPure DNA Extraction Kit is optimized to maximize the recovery of genomic DNA from 10 or more cells captured by LCM. The kit comes with reagents and protocol tested to ensure complete extraction of DNA from LCM samples prepared with any standard tissue preparation procedure. DNA prepared using the kit is *PCR-ready* and needs no additional purification to perform amplification.

**HistoGene® LCM Immunofluorescence Staining and Dehydration Kits**

The HistoGene LCM Immunofluorescence Staining and Dehydration Kits are the only kits designed to enable retrieval of high-quality RNA from immunofluorescently stained frozen tissue. They enable 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.

---

## II. Kit Components

---

### A. Reagents and Supplies in Kit

The Arcturus® HistoGene® LCM Frozen Section Staining Kit comes with the following items.

Description	Unit
Xylene	500 ml
100% Ethanol	500 ml
95% Ethanol	500 ml
75% Ethanol	1000 ml
Distilled Water	1000 ml
Staining Solution	8 ml
Plastic Slide Jars	10 jars
LCM Slides	72 slides

---

## III. Preliminary Steps

---

### A. Material and Protocol Review

To get the most from your Arcturus® HistoGene® LCM Frozen Section Staining Kit, take a few moments to examine the components of the kit and read through the information in this section and the next one (sections III and IV).

---

### B. Recommendations for RNase-Free Technique

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

- Wear disposable gloves and change them frequently.
- Use RNase-free solutions, glassware and plasticware.
- Do not re-purify kit components. They are certified Nuclease Free.
- Wash scalpels, tweezers and forceps with detergent and bake at 210°C for four hours before use.
- Use RNase AWAY® solution (Life Technologies) according to the manufacturer's instructions on the horizontal staining rack and any other surfaces that may come in contact with the sample.



## 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 Arcturus® HistoGene® LCM Frozen Section Staining Kit:

### 1. Equipment

- Cryostat with disposable microtome blades
- Fume hood
- 70°C freezer
- 200 µl handheld pipettor
- Desiccator
- Scalpels
- Tweezers
- Cover glass forceps
- Microslide box, plastic (VWR Cat. # 48444-004)
- Optional: Horizontal staining tray (Sigma Cat. # H6644)

### 2. Materials

- Disposable gloves
- RNase-free pipet tips
- Tissue-Tek® OCT compound (VWR Cat. # 25608-930)
- Tissue-Tek® Cryomold (VWR Cat. #25608-916)
- Dry ice
- Detergent (Fisher Scientific, Cat. # 04-355)
- RNase AWAY solution (Life Technologies, Cat. # 10328-011)
- 100% ethanol
- Kimwipes® or similar lint-free towels
- Desiccant (VWR Cat. # 22890-900)

---

## IV. Protocol

---

### A. Specimen Freezing

1. Place cryomold on dry ice and place a thin layer of OCT on bottom of cryomold.
2. Collect dissected tissue specimen.
3. Place tissue specimen in desired orientation in cold cryomold from step 1.
4. Add OCT to cold cryomold until specimen is completely covered.
5. Wait for OCT to solidify completely.
6. Proceed to slide preparation or store frozen specimen in the cryomold in a  $-70^{\circ}\text{C}$  freezer.



*For best RNA preservation, freeze tissue specimens immediately after dissection.*



*Wear clean disposable gloves throughout the Specimen Freezing procedure. Use clean RNase-free instruments.*

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


### B. Slide Preparation

1. Pre-cool the cryostat to the temperature recommended by the manufacturer for the specimen you are preparing.
2. Remove and discard old microtome blade. Wipe down the knife holder and antiroll plate in the cryostat with 100% ethanol to avoid sample cross-contamination. Do **not** use the 100% ethanol solution provided in the HistoGene Frozen Section Staining Kit for this step.
3. Install a new disposable microtome blade in the cryostat.
4. Set cutting thickness to 8  $\mu\text{m}$ .
5. Place a microslide box on dry ice near the cryostat.



*Wear clean disposable gloves throughout the Slide Preparation procedure.*


- 
6. Transfer the cryomold containing the specimen from the  $-70^{\circ}\text{C}$  freezer to the cryostat, transporting on dry ice if necessary.
  7. Wait a minimum of 10 minutes for the specimen to equilibrate with the temperature of the cryostat.
  8. Mount specimen to specimen holder with OCT. Cut  $8\ \mu\text{m}$  sections.
  9. Mount sections towards the center of a room temperature LCM microslide. Place slide immediately into microslide box on dry ice. Do not allow slide to dry at room temperature.
  10. Discard slides with folded or wrinkled sections. If cutting more than one specimen, use a new disposable microtome blade for each one. In addition, wipe down knife holder and anti-roll plate with 100% ethanol in between each specimen to avoid cross-contamination.
  11. Proceed immediately to the “Staining and Dehydration” segment of the protocol or store at  $-70^{\circ}\text{C}$  for up to two months.

 *Frequent cycling of the tissue block from  $-70^{\circ}\text{C}$  to  $-20^{\circ}\text{C}$  for cryosectioning may accelerate RNA degradation. For best results, cut and mount a sufficient number of sections for two months' use during one cryosectioning session. Store the mounted sections at  $-70^{\circ}\text{C}$  until needed.*

---

## C. Staining and Dehydration

1. Label seven plastic slide jars as follows:
  - a. 75% ethanol
  - b. distilled water
  - c. distilled water
  - d. 75% ethanol
  - e. 95% ethanol
  - f. 100% ethanol
  - g. xylene
2. Using the LCM Certified solutions provided with your HistoGene Frozen Section Staining Kit, fill the labeled plastic slide jars with 25 ml of the appropriate solution.
3. If using a horizontal staining tray, clean surface with RNase Away.
4. Remove a maximum of four slides from the slide box on dry ice or from the  $-70^{\circ}\text{C}$  freezer, and place them on a clean Kimwipe towel (or similar lint-free towel) and allow to thaw for no more than 30 seconds.
5. Place the slides in plastic slide jar “a” containing 75% ethanol for 30 seconds. Use cover glass forceps to transfer slides from jar to jar.
6. Transfer the slides to plastic slide jar “b” containing distilled water for 30 seconds.
7. Place the slides on a Kimwipe towel or a horizontal staining tray.
8. Using an RNase-free pipette tip, apply approximately 100  $\mu\text{l}$  of the HistoGene Staining Solution so that it covers the section. Stain for 20 seconds.
9. Place the slides in plastic slide jar “c” containing distilled water for 30 seconds.
10. Transfer the slides to plastic slide jar “d” containing 75% ethanol for 30 seconds.

 Carry out the “Staining and Dehydration” segment of the protocol in a hood. Wear clean disposable gloves. Divide the work batchwise, with a maximum of four slides per batch. Change all solutions in the plastic slide jars between each batch of slides to avoid cross contamination. Do not reuse solutions. Do not transfer solutions back into their original bottles. If you plan to reuse jars, discard all solutions upon completion of the staining and clean them (see Section VI).

- 
11. Transfer the slides to plastic slide jar “e” containing 95% ethanol for 30 seconds.
  12. Transfer the slides to plastic slide jar “f” containing 100% ethanol for 30 seconds.
  13. Transfer the slides in to plastic slide jar “g” containing xylene for five minutes.
  14. Place the slides on a Kimwipe to towel dry in the hood for five minutes.
  15. Place all slides in a desiccator or slide box containing fresh desiccant.
  16. Remove one slide and perform LCM, keeping the remainder in the desiccator until ready for LCM.
  17. Discard all used staining and dehydration solutions according to your procedures.

## V. Troubleshooting

---

### A. Targeted cells do not lift from the slide during LCM

1. The sample may contain residual water. Ensure that the ethanol solutions are fresh. Ethanol is hygroscopic. Keep the ethanol bottles tightly capped, and do not pour ethanol solutions until you are ready to use them. If you suspect that the 100% ethanol solution has absorbed water, purchase a new bottle.
  2. The sample may have dried between protocol steps. Carry out the “Staining and Dehydration” segment of the protocol at a steady pace.
- 

### B. RNA cannot be recovered from the sample

1. The sample starting material may contain poor quality RNA. Freeze the sample immediately following dissection, and take care to use RNase-free technique.
  2. RNA may become degraded during RNA isolation. Wear gloves; use RNase-free technique and RNase-free instruments and reagents. Wipe down the Arcturus<sup>®</sup> Laser Capture Microdissection System with RNase AWAY prior to use.
  3. RNA may not be fully extracted and isolated from cells on the LCM cap. Use the Arcturus PicoPure RNA Isolation Kit or another guanidinium extraction method. Perform RNA extraction immediately after LCM to ensure complete extraction and optimum recovery of RNA.
  4. The starting material quantity may be insufficient. Use at least 10 captured cells. The isolation procedure used in the Arcturus<sup>®</sup> PicoPure<sup>®</sup> RNA Isolation Kit provides reproducible recovery of RNA from the equivalent of 10 cells (200 pg) as assayed by RT-PCR.
-

---

## VI. Appendix

---

### A. Related Protocols

#### 1. Cleaning Plastic Slide Jars

The plastic slide jars provided with the HistoGene Frozen Section Staining Kit can be reused, but must be cleaned between each batch of slides. Rinse jars with 100% ethanol, followed by distilled water, then treat with RNase AWAY solution according to the manufacturer's protocol. Rinse jars thoroughly with nuclease-free water and allow to dry completely in the hood. Do not use jars to store solutions.

#### 2. RNA Extraction and Isolation

A complete extraction and purification method for preparing RNA from captured cells is available in the PicoPure RNA Isolation Kit (Applied Biosystems Cat. #KIT0204). The kit comes with a detailed, validated protocol and RNase-free materials. The kit materials and protocol produce total cellular RNA in a 10  $\mu$ l volume ready for analysis.

#### 3. Checking RNA Integrity with RT-PCR and Gel Analysis of mRNA from LCM Samples

- a. The following RT-PCR protocol can detect the presence of specific mRNA in samples prepared from small numbers of cells. Quantitative fluorescence imaging confirms the size and relative abundance of mRNA transcripts from tissues prepared using the HistoGene Frozen Section Staining Kit and the PicoPure RNA Isolation Kit.

- 
- b. Mix 10  $\mu$ l of RNA obtained from 500 cells captured by LCM with 10  $\mu$ l Reverse Transcriptase master mix for cDNA synthesis as described in the Sensiscript Reverse Transcriptase Handbook (Qiagen) using oligo-dT primers and RNase inhibitor (Life Technologies, Cat. # 18418-012 and Cat. # 10777-019). Carry out the reverse transcriptase reaction for one hour at 37°C followed by a five minute denaturation step at 95°C as described by the manufacturer.
- c. Perform PCR using primer sets for three different abundance level genes as provided in the Stratagene Control RT-PCR Primers Kit (Cat. # 720170). Using a 0.5 ml thin-walled tube, add 2  $\mu$ l of the synthesized cDNA solution to 23  $\mu$ l of master mix containing forward and reverse primers at a final concentration of 200 nM along with one Ready-to-Go<sup>®</sup> bead (GE Healthcare). Denature samples at 95°C for five minutes, then amplify using the following thermal cycle parameters for 35 cycles, with a final extension of 72°C:

	95°C	15 seconds
primer anneal temp.		30 seconds
	72°C	1 minute

- d. Add gel loading buffer to the PCR sample and separate 20  $\mu$ l of the mixture on a 6% Novex acrylamide gel (Invitrogen, Cat. # EC6265). Stain the gel with SYBR<sup>®</sup> Gold Nucleic Acid Gel Stain (Molecular Probes, Cat. # S11494), then visualize on a FluorImager system (Molecular Dynamics).



---

#### **4. Tissue Scrape Protocol for Verifying RNA Quality Using the Acturus® PicoPure® RNA Isolation Kit**

Applied Biosystems recommends verifying the integrity of RNA in the tissue sample before proceeding with staining and Laser Capture Microdissection (LCM) procedures. This enables you to understand the quality of the RNA in the experimental sample before proceeding with further downstream processing. This protocol is recommended for all new frozen tissue samples.

The protocol involves preparing and dehydrating a tissue section, then scraping the entire tissue section into a 0.5 ml tube. RNA is then extracted from the sample using a modified version of the Arcturus® PicoPure® RNA Isolation Kit (Catalog # KIT0204) protocol for larger amounts of tissue. Finally, the Lab-on-a-Chip System (Agilent) or a gel can be used to assess 28S and 18S ribosomal RNA integrity. If ribosomal bands are detected, then the sample contains viable RNA and is therefore a good candidate for LCM. If the ribosomal RNA bands are faint or not present, then the sample may contain degraded RNA.

For more information, visit [www.appliedbiosystems.com](http://www.appliedbiosystems.com), or call Technical Support at 1-800-831-6844 option 5.



Part Number 12294-00 Rev. C 07/2010

---



**Headquarters**

5791 Van Allen Way  
Carlsbad, CA 92008 USA  
Phone 760.603.7200  
[www.lifetechnologies.com](http://www.lifetechnologies.com)

**Technical Resources and Support**

For the latest technical resources and support information for all locations, please refer to our Web site at [www.appliedbiosystems.com](http://www.appliedbiosystems.com)