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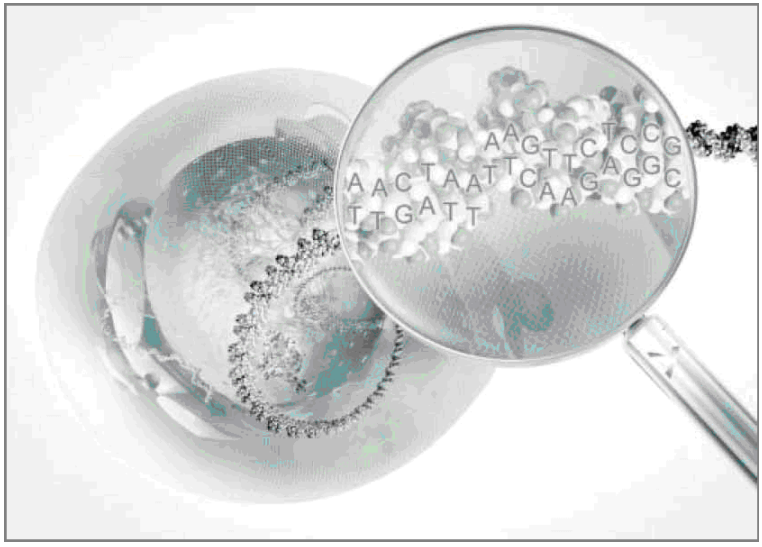
Arcturus® PicoPure® DNA Extraction Kit

User Guide



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TRADEMARKS

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1. Introduction

1.1. BACKGROUND

The Arcturus® PicoPure® DNA Extraction Kit provides a fast and easy genomic DNA extraction procedure. Conveniently packaged reagents for preparing PCR-ready DNA from animal tissue and cell samples prepared using a variety of methods are included. No organic extractions or spin columns are needed, enabling very high DNA recovery from samples and single-tube DNA extraction and subsequent amplification.

This streamlined method makes the kit ideal for processing small samples, of as few as ten cells, where maximum DNA recovery is critical. However, the extraction procedure is equally well suited for larger samples of up to several milligrams of tissue or cell pellets. The kit uses an optimized Proteinase K extraction procedure, and features lyophilized, premium-quality enzyme in convenient aliquots. These are freshly reconstituted before each use for optimal enzymatic activity. DNA extracted using the kit is ready for use in endpoint or quantitative real-time PCR assays.

DNA can be extracted from tissue prepared using a wide variety of preparation methods. Superior results can be obtained from paraffin-embedded, formalin-fixed tissue sections, frozen tissue sections, ethanol-fixed cells, and fresh or previously frozen cell pellets. The method is not designed for DNA extraction from whole blood, plant, or fungal samples.

A General DNA extraction procedure for processing non-microdissected samples is provided in Section 4.4., *"DNA Extraction Protocol: Cell Pellet and Tissue Section Samples"*. Procedures for processing small samples obtained using Arcturus® Laser Capture Microdissection (LCM) Systems are also provided in Section 4.5., *"DNA Extraction Protocol: CapSure® HS LCM Cap Samples"* and Section 4.6., *"DNA Extraction Protocol: CapSure® Macro LCM Cap Samples"*. Separate procedures are provided for using CapSure® HS or Macro LCM Caps.

The kits are tested to ensure the absence of DNA and DNases, and for compliance with other quality measurements. For example, each lot of Proteinase K is reconstituted in Reconstitution Buffer and tested for protease activity. In addition, functional testing is performed according the protocols described in this user guide.

⚠ **WARNING:** The Arcturus® PicoPure® DNA Extraction Kit has been used successfully with most tissue specimens, including archived tissue samples. For PCR, the kit has been tested using Taq polymerase from a variety of suppliers. If you intend to use the kit with cells derived from archived tissue samples, or if you have any other questions about

the compatibility of the kit with other substrates or materials, please contact Technical Support before you begin by calling 1-800-831-6844 option 5.

1.2. STORAGE AND STABILITY

Arcturus® PicoPure® DNA Extraction Kit reagents are shipped at room temperature. Upon receipt, the kits should be stored at room temperature in a cool dry place. Proteinase K should be used immediately after reconstitution. Freeze thawing is not recommended and will decrease the stability and shelf life of the enzyme.

⚠ **WARNING:** Reconstitution Buffer contains sodium azide, a potent toxin which may be fatal if swallowed or absorbed through skin. Use appropriate precautions.

1.3. SAFETY DATA SHEETS

Chemical manufacturers supply current Safety Data Sheets (SDSs) with shipments of hazardous chemicals to new customers. They also provide SDSs with the first shipment of a hazardous chemical to a customer after an SDS has been updated. SDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new SDS packaged with a hazardous chemical, be sure to replace the appropriate SDS in your files.

The SDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain SDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **SDS**.
2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

For the SDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

1.4. RELATED PRODUCTS

Arcturus® HistoGene® LCM Frozen Section Staining Kit, PicoPure® RNA Isolation Kits, and RiboAmp® RNA Amplification Kits are optimized for use in LCM procedures. For an updated list of available kits, please contact Technical Support at 1-800-831-6844 option 5.

1.4.1. RELATED PRODUCTS

Catalog#	Product
LCM0211	CapSure® Macro LCM Caps
LCM0214	CapSure® HS LCM Caps
LCM0213	CapSure® HS LCM Caps Starter Pack

2. Components

2.1. REAGENTS AND SUPPLIES

The Arcturus® PicoPure® DNA Extraction Kit comes with the following items:

→ Proteinase K 10 x 0.5 ml Vials

→ Reconstitution Buffer 1 x 2.0 ml Vials

⚠ **WARNING:** The Arcturus® PicoPure® DNA Extraction Kit has been optimized for use with the reagents provided. Substitution of other digestion enzymes or buffers may yield unsatisfactory results.

3. Equipment and Materials

3.1. EQUIPMENT AND MATERIALS

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

3.1.1. EQUIPMENT

→ Microcentrifuge (capable of 4000 x g with microfuge tube rotor)

→ Standard laboratory incubator set at 65° C

→ Heating block for 0.5 ml microcentrifuge tubes (95° C)

→ Hand-held pipettor

→ LCM Cap Insertion Tool (provided with the Arcturus® LCM System)

If using CapSure® HS LCM Caps:

→ ExtracSure™ Device (Cat. # 0208)

→ Alignment Tray (Cat. # LCM0504)

→ Incubation Block (Cat. # LCM0505)

→ Clean forceps

→ Gloves

3.1.2. MATERIALS

→ LCM Captured Cells on either CapSure® Macro LCM Cap (Cat. # LCM 0211) or CapSure HS LCM Cap (Cat. # LCM 0214)

→ Nuclease-free pipette tips

→ Ice in ice bucket

→ 0.5 ml microcentrifuge tubes (either standard or thin-walled). CapSure Macro LCM Caps, CapSure HS LCM Caps, and the ExtracSure Device are compatible with 0.5 ml autoclaved thin-walled reaction tubes (Applied Biosystems; N8010611).

4. Protocol

4.1. OVERVIEW

To ensure best results, please take a few minutes to review this protocol before starting and to organize the equipment and materials needed to perform the protocol.

The Arcturus® PicoPure® DNA Extraction Kitextracts DNA from cells captured using either CapSure® Macro LCM Caps or CapSure HS LCM Caps. We recommend using 100 cells as a routine starting sample until amplification conditions are optimized for fewer cells.

The kit can also be used to extract DNA from small sections of tissue and from cultured cells.

The kit includes sufficient material for 30 extractions when using CapSure Macro LCM Caps and 150 extractions when using CapSure HS LCM Caps.

4.2. TIME REQUIREMENTS

Total processing time depends on the tissue source. Extraction from non-formalin fixed samples is very efficient and requires only three hours incubation time. Due to the cross linking that occurs during fixation, DNA extraction from formalin fixed tissue requires a minimum of 16 hours incubation time.

4.3. BEFORE YOU BEGIN

1. Pre-heat incubator to 65° C. If using CapSure HS Caps with the ExtracSure Device, place heating block in incubator so it can be at the proper temperature when samples are ready for extraction.
2. Centrifuge one vial of Proteinase K for 30 seconds at 1000 x g prior to addition of Reconstitution Buffer.
3. Set an ice bucket with ice at your benchtop work area.

4.4. DNA EXTRACTION PROTOCOL: CELL PELLET AND TISSUE SECTION SAMPLES

The following protocols are recommended for extracting DNA from cell pellets or formalin-fixed, paraffin-embedded tissue sections.

1. Pipette 155 µl of Reconstitution Buffer into one vial of Proteinase K. Gently vortex the tube to mix the reagents and complete dissolve the reagent. Immediately place the tube on ice and use as soon as possible. Excessive mixing may denature the Proteinase K. This Proteinase K / Reconstitution buffer mix will now be referred to as "Extraction Solution".
2. Follow the steps for cells or non-fixed and fixed tissue, respectively.

   Cells

   a Pellet 1 x 105–1 x 106 cells in a 1.5 ml microcentrifuge tube.

   b Remove the supernatant.

   c Add 150 µl of Extraction Solution to the tube.

   d Vortex gently to mix cells thoroughly.

   e Incubate sample at 65° C for three hours.

   f Place sample tubes at 95° C for ten minutes to inactivate Proteinase K.

   Fixed tissue sections

   a For paraffin-embedded tissue, remove paraffin by immersing the tissue section in approximately 15 ml of freshly poured xylene, two times, each for five minutes. If the tissue was fixed in ethanol, remove the tissue from the ethanol, and air-dry the tissue for five minutes.

   b Carefully scrape 1.5–2 µg of tissue into a 1.5 ml microcentrifuge tube.

   c Add 150 µl of Extraction Solution to the tube.

   d Vortex gently to mix thoroughly.

   e Incubate at 65° C for up to 24 hours.

   f Place sample tubes at 95° C for ten minutes to inactivate Proteinase K.
3. Proceed directly to PCR or store digested samples at –20° C.

4.5. DNA EXTRACTION PROTOCOL: CAPSURE® HS LCM CAP SAMPLES

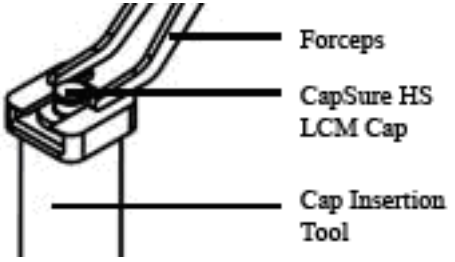
1. Pipette 155 µl of Reconstitution Buffer into one vial of Proteinase K. Gently vortex the tube to mix the reagents and immediately place the tube on ice. Completely dissolve reagent, excessive mixing can denature Proteinase K. This Proteinase K / Reconstitution Buffer mix will now be referred to as "Extraction Solution".

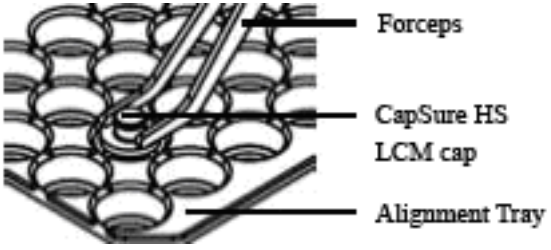
⚠ **WARNING:** Use Proteinase K extraction solution as quickly as possible following reconstitution. Discard any unused Proteinase K extraction solution.

⚠ **WARNING:** For best results, preheat Incubation Block in 65° C incubator.

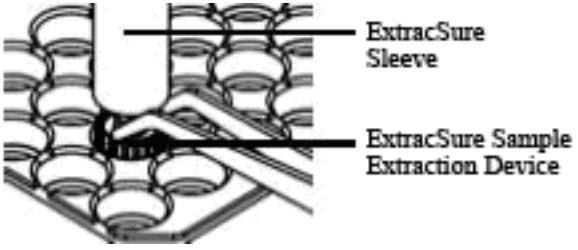
2 Assemble the ExtracSure™ Device onto the CapSure HS LCM Cap.

a Using clean forceps, remove the cap from the Cap Insertion Tool and place into the Alignment Tray (sample facing up). Make sure that the cap snaps securely and lays flat on the bottom of the opening of the Alignment Tray.

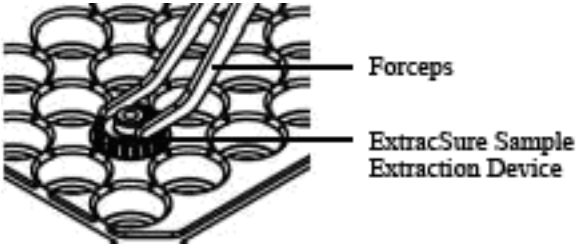




b Position the ExtracSure Device over the cap using clean forceps. The fill-port should be facing up.



c Using forceps, push the ExtracSure Device down onto the device cap until it snaps securely into place. Use forceps to ensure that the cap is firmly attached to the ExtracSure Device.

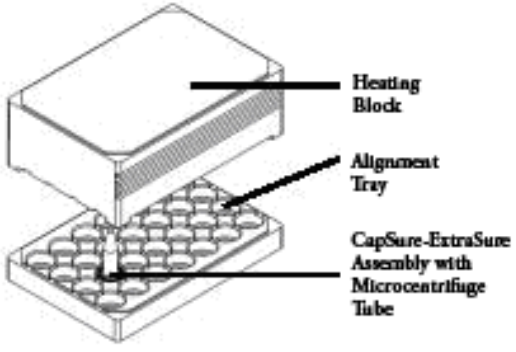


3 Pipette 10 µl of extraction solution into the microchamber formed by the assembled ExtracSure Device and CapSure HS LCM Cap.

4 Using gloved hands, place a 0.5 ml thin-walled reaction tube over the ExtracSure device.

5 Cover the assembly in the Alignment Tray with the Incubation Block and incubate it for the correct time period according to the following table:

Sample Type	Incubation time (65° C)
Formalin Fixed/Paraffin embedded	>16 hours
Non formalin Fixed/paraffin Embedded	≅ 3 hours
Frozen	≅ 3 hours
Cytospin & Cell Smears	≅ 3 hours



6 After incubation, remove the assembled CapSure HS LCM Cap and ExtracSure Device from the incubator, place the assembly into a microcentrifuge and centrifuge for one minute at 4,000 x g.

7 Separate the CapSure HS LCM Cap and ExtracSure Device. Close the microcentrifuge tube containing the extract and heat to 95° C for ten minutes in a heating block to inactivate the Proteinase K. Cool the sample to room temperature.

8 Samples are now ready for PCR analysis. Additional purification should not be required.

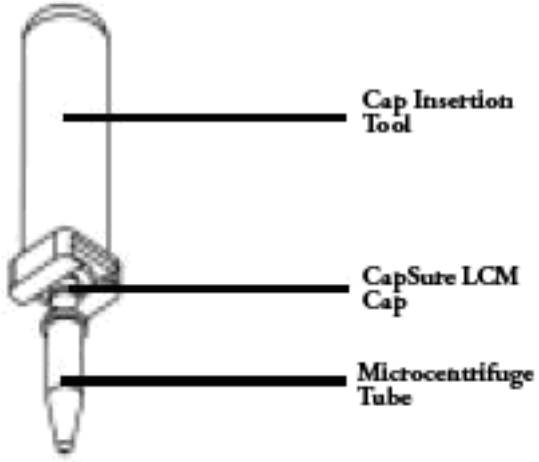
⚠ **WARNING:** Some samples may benefit from longer incubation times, or higher reaction concentrations of Proteinase K. See Chapter 5, *"Troubleshooting"*. For specific advice, please contact Technical Support at 1-800-831-6844 option 5.

4.6. DNA EXTRACTION PROTOCOL: CAPSURE® MACRO LCM CAP SAMPLES

1 Pipette 155 µl of Reconstitution Buffer into one vial of Proteinase K. Gently vortex the tube to mix the reagents and place the tube on ice immediately. Completely dissolve reagent, excessive mixing may denature Proteinase K. This Proteinase K / Reconstitution Buffer mix will now be referred to as "Extraction Solution".

2 Pipette 50 µl of Proteinase K extraction solution into a 0.5 ml microcentrifuge tube.

3 Insert the CapSure Macro LCM Cap with LCM captured cells into the microcentrifuge tube using the Cap Insertion Tool.



⚠ **WARNING:** Use extraction solution as quickly as possible following reconstitution. Discard any unused Proteinase K extraction solution.

4 Invert the microcentrifuge tube with the inserted CapSure Macro LCM Cap and shake down the 50 µl volume of extraction solution until it completely covers the inside surface of the CapSure Macro LCM Cap.

5 Incubate the inverted tube at 65° C for the correct time period according to the following table:

Sample Type	Incubation Time (65° C)
Formalin Fixed/Paraffin Embedded	> 16 hours
Non Formalin Fixed/Paraffin Embedded	≅ 3 hours
Frozen	≅ 3 hours
Cytospin & Cell Smears	≅ 3 hours

6 After incubation, remove the tubes from the incubator, place them in a microcentrifuge and centrifuge for one minute at 1,000 x g.

7 Remove the CapSure Macro LCM Cap. Close the microcentrifuge tube containing the extract and heat to 95° C for ten minutes in a heating block to inactivate the Proteinase K. Cool the sample to room temperature.

8 Samples are now ready for PCR analysis. Additional purification should not be required.

5. Troubleshooting

5.1. INCOMPLETE DIGESTION OF CELLS OFF THE CAPSURE® MACRO LCM CAP

1. Proteinase K may be inactive. Always use freshly reconstituted Proteinase K. Reconstituted aliquots of Proteinase K (extraction solution) can lose enzymatic activity during storage.
2. Proteinase K may have become inactive due to enzyme autodigestion during long incubation periods. For samples requiring prolonged incubation, pipette an additional 10 µl of freshly reconstituted Proteinase K extraction solution into the reaction chamber following the first 24 hours of incubation.
3. Samples with large amounts of connective tissue may require longer incubations with Proteinase K for complete digestion.
4. Proteinase K is absent from the sample extraction buffer. Be sure to add Reconstitution Buffer to a fresh vial of Proteinase K before use.

5.2. BUFFER LEAKS DURING INCUBATION

1. If buffer leaks from microcentrifuge tube, ensure that CapSure Macro LCM Cap is properly inserted into the microcentrifuge tube.
2. If buffer leaks from ExtracSure Device, ensure that the device is properly assembled onto the CapSure HS LCM Cap. Do not remove assembled device from Alignment Tray before incubation.

5.3. UNABLE TO AMPLIFY DNA

1. Template DNA quantity is insufficient. Increase the number of cells in starting sample or the amount of lysate transfered to amplification reaction.
2. Proteinase K was not inactivated. If Proteinase K was not completely inactivated prior to addition of PCR reagents, Proteinase K may digest the Polymerase. Following Proteinase K digestion, heat extracted sample for ten minutes above 95° C to inactivate Proteinase K.
3. PCR parameters are not optimized. Make sure PCR primer sequences are correct for target gene amplification. Verify that PCR primers are present in the proper concentrations for the reaction conditions. Ensure that the annealing temperature has been optimized.

4 PCR inhibitors may be present in sample. PCR inhibitors (heme, melanin, certain stains in high concentration such as hematoxylin) may have carried over from tissue preparation. Purify the DNA sample further before PCR.

5 PCR priming is non-specific. Optimize primer concentration and reaction conditions.

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Headquarters
5791 Van Allen Way
Carlsbad, CA 92008 USA | Phone 760.603.7200
www.lifetechnologies.com

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