applied biosystems
life technologies

Laser Capture Microdissection Cells-to-C_T™ Kit

Low-Input RNA Samples

Catalog Number A26764

Pub. No. MAN0010915 **Rev.** A

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Note: For safety and biohazard guidelines, refer to the "Safety" appendix in the *Arcturus XT™ Laser Capture Microdissection System User Guide* (Pub. no. 0112-0153), the *Single Cells-to-C_T™ Kit Protocol* (Pub. no. 4458356), and your Applied Biosystems® Real-Time PCR System user documentation. Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

About the kit

The Laser Capture Microdissection Cells-to-C_T™ Kit (Cat. no. A26764) provides a complete solution for measuring relative expression of RNA transcripts from frozen tissue obtained by laser microdissection. The kit features a validated qRT-PCR workflow optimized for maximum sensitivity to yield reproducible results from samples consisting of as few as 10 cells. The kit is designed for use with the Arcturus XT™ Laser Capture Microdissection System using either CapSure® HS Caps or CapSure® Macro Caps.

Workflow

The following workflow illustrates how to prepare and analyze samples using the Laser Capture Microdissection Cells-to-C_T™ Kit.

Laser capture microdissection
▼
Cell lysis/RNA extraction
▼
Reverse transcription
▼
cDNA pre-amplification
▼
Real-time PCR analysis

Obtain related documentation

This document incorporates procedures from the *Arcturus XT™ Laser Capture Microdissection System User Guide* (Pub. no. 0112-0153) and the *Arcturus® Histogene™ Frozen Section Staining Kit User Guide* (Pub. no. 12294-00). Where noted, the procedures in this guide have been condensed and lack the level of detail found in the source documentation. For detailed explanation of an abbreviated procedure, refer to the source document for the reagent, kit, or instrument of interest.

To obtain any of the source documents in PDF format from the Life Technologies website, go to **www.lifetechnologies.com/support**, then search for the publication number of interest or the catalog number of the related kit.

Materials and equipment

IMPORTANT! Store all kits and consumables under the recommended conditions and in an upright position.

The Laser Capture Microdissection Cells-to-C_T™ Kit includes the materials shown below. Upon arrival, inspect all consumables and contact Life Technologies if any of the products have been damaged during shipping.

Contents and Storage

Component	Quantity	Storage
CapSure® Macro LCM Caps or CapSure® HS Caps/ ExtracSure™ Devices	48 caps 32 caps	Room temp.[1]
Single Cell Lysis Solution	0.5 mL	2 to 6°C
TaqMan® Gene Expression Master Mix	5.0 mL	
Single Cell DNase I	50 µL	-30°C to -10°C
Single Cell Stop Solution	50 µL	
Single Cell VILO™ RT Mix	150 µL	
Single Cell SuperScript® RT	75 µL	
Single Cell PreAmp Mix	265 µL	

[1] Room temperature (15°C to 30°C), away from direct light.

life technologies
A Thermo Fisher Scientific Brand

Materials and equipment not provided

The following table lists required materials and equipment that are not provided with the Laser Capture Microdissection Combo Kits. Where noted, supplies are available from Major Laboratory Suppliers (MLS).

Material	Source
Arcturus® Laser Capture Microdissection System	Thermo Fisher[1]
Cryostat	Thermo Fisher
GeneAmp® Thin-Walled Reaction Tubes, with domed cap, 0.5 mL	Thermo Fisher (Cat. no. N8010611)
Histogene™ LCM Frozen Section Staining Kit	Thermo Fisher (Cat. no. KIT0401)
Microcentrifuge	MLS
Nuclease-free water	Thermo Fisher
PEN Membrane Glass Slides	Thermo Fisher (Cat. no. LCM0522)
Pipette tips, nuclease-free	Thermo Fisher
Pipettors	MLS
Real-time PCR Instrument	Thermo Fisher
MicroAmp® Optical Reaction Plates and Seals[2]	Thermo Fisher
TaqMan® Gene Expression Assays	Thermo Fisher
TE Buffer (1X)	Thermo Fisher
Thermal cycler	Thermo Fisher
Vortexer	MLS
Additional materials required if using CapSure® HS Caps and ExtracSure™ Devices	
Alignment Tray for Sample Extraction from CapSure® HS Caps	Thermo Fisher (Cat. no. LCM0504)

[1] Go to www.lifetechnologies.com for product information.

[2] Appropriate for your real-time PCR instrument.

Perform Laser Capture Microdissection

Collect the desired sample(s) from frozen tissue sections prepared according to the *Arcturus® Histogene™ Frozen Section Staining Kit User Guide* (Pub. no. 12294-00).

If using...	Collect...
CapSure® Macro LCM Caps	regions up to 3 mm in diameter
CapSure® HS Caps and ExtracSure™ Devices	10-100 cells

Note: Refer to the *Arcturus XT™ Laser Capture Microdissection System User Guide* (Pub. no. 0112-0153) for general laser capture microdissection information.

Perform the RNA Extraction

Perform the appropriate procedure below depending on the type of caps used to obtain the microdissected samples (CapSure® HS Caps or CapSure® Macro Caps).

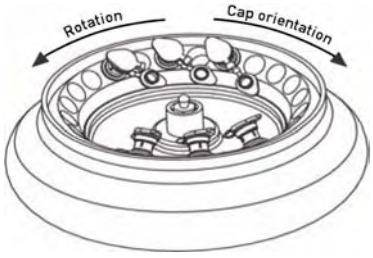
Perform RNA extraction using CapSure® HS Caps

IMPORTANT! If you are using CapSure® Macro Caps, see "Perform RNA extraction using CapSure Macro Caps" on page 3.

1. Prepare the lysis buffer and tubes:
   a. Label a 0.5-mL GeneAmp® Thin-Walled Reaction Tube for each microdissected sample that you are preparing.
   b. Prepare lysis buffer for your samples by combining the following components in a reaction tube. Scale the volumes appropriately for the number of samples that you are preparing.

Component	Volume per cap
Single Cell Lysis Solution	9 µL
Single Cell Dnase I	1 µL
Total Lysis mix for each HS Cap	**10 µL**

2. Dispense the lysis buffer and incubate the samples:
   a. Capture the desired cell(s) and assemble the CapSure® HS Caps and ExtracSure™ Extraction devices as explained in the *Arcturus XT™ Laser Capture Microdissection System User Guide* (Pub. no. 0112-0153).
   b. Pipette 10 µL of prepared lysis buffer into the buffer well of the cap assembly of each tube.
   c. Incubate the tube assemblies at room temperature for 5 minutes.
3. Add 1 µL Stop Solution to each tube.
4. Incubate the capped tubes at room temperature for 2 minutes, then place them on ice.
5. Attach a 0.5-mL GeneAmp® Thin-Walled Reaction Tube to the top of each CapSure®/ ExtracSure™ assembly.
6. Centrifuge the tube assemblies at 800 x g for 2 minutes to collect the cell extract at the bottom of each tube.



7. Remove and discard the CapSure®/ ExtracSure™ assembly from each tube containing cell lysate, then cap it.

STOPPING POINT If necessary, the lysates can be stored at -70°C for up to one month.

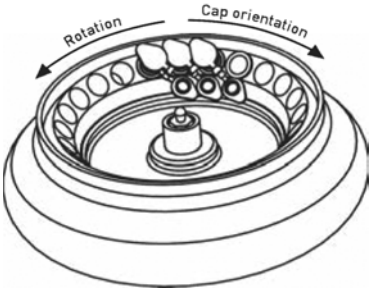
Perform RNA extraction using CapSure® Macro Caps

IMPORTANT! If you are using CapSure® HS Caps, see "Perform RNA extraction using CapSure HS Caps" on page 2.

1. Prepare the lysis buffer and tubes:
   a. Label a 0.5-mL GeneAmp® Thin-Walled Reaction Tube for each microdissected sample that you are preparing.
   b. Prepare lysis buffer for your samples by combining the following components in a reaction tube. Scale the volumes appropriately for the number of samples that you are preparing.

Component	Volume per cap
Single Cell Lysis Solution	50 µL
Single Cell Dnase I	5 µL
Total Volume	**55 µL**

2. Dispense the lysis buffer and incubate the samples:
   a. Transfer 55 µL of prepared lysis buffer to each labeled GeneAmp® Reaction Tube.
   b. Using the CapSure® Insertion Tool, insert a CapSure® Macro Cap into each tube. After applying each cap, firmly press down on the insertion tool to ensure a tight and even seal between the cap and tube.
   c. Invert the cap-tube assemblies so that the Lysis Buffer comes into contact with the microdissected cells on the CapSure® Macro Caps. If necessary, tap the bottom of each tube to ensure that buffer completely covers the cap surfaces.
   d. Incubate the cap-tube assemblies at room temperature for 5 minutes.
3. Centrifuge the cap-tube assemblies at 800 x g for 2 minutes to collect the cell extract at the bottom of each tube.



4. Remove the CapSure® Macro Cap from each microcentrifuge tube and discard it.
5. Add 5 µL of Single Cell Stop Solution to each microcentrifuge tube containing cell lysate, then cap it.
6. Incubate the capped tubes at room temperature for 2 minutes, then place them on ice.

STOPPING POINT If necessary, the lysates can be kept on ice for up to 30 minutes or stored at -20°C.

Note: The total sample volume of each cell lysate is 60 µL.

Perform reverse transcription

Perform reverse transcription of the cell lysates as follows. For safety guidelines and kit usage information, refer to the *Single Cells-to-C_T™ Kit Protocol* (Pub. no. 4458356).

1. Transfer 10 µL of cell lysate per sample to individual wells of a 96-well plate.

   Note: Freeze the remaining lysate for future experiments.
2. Prepare sufficient RT reaction mix for all samples.

Component	Volume per reaction
Single Cell VILO™ RT Mix	3.0 µL
Single Cell SuperScript® RT	1.5 µL
Total RT reaction Mix	**4.5 µL**

3. Add 4.5 µL to each cell lysate in the reaction plate.
4. Seal the reaction plate, then perform reverse transcription using a thermal cycler:

Stage	Temperature	Time
Hold	25°C	5 min
Hold	42°C	60 min
Hold	85°C	10 min

STOPPING POINT The cDNA samples can be stored at -20°C.

Note: The total volume of each cDNA sample is 15.5 µL.

Perform cDNA preamplification

Perform a preamplification of the cDNA product as follows. For safety guidelines and kit usage information, refer to the *Single Cells-to-C_T™ Kit Protocol* (Pub. no. 4458356).

1. Pool the TaqMan® Gene Expression Assays for the targets of interest, then dilute the pooled assays using 1X TE Buffer (pH 8.0) so that each assay achieves a final concentration of 0.2X.
2. Prepare sufficient PreAmp Reaction Mix, then add 11 µL to each reverse-transcribed sample:

Component	Volume per reaction
Single Cell PreAmp Mix	5 µL
TaqMan® Gene Expression Assays, pooled (0.2X)	6 µL
Total PreAmp Reaction Mix	**11 µL**

3. Perform preamplification using a thermal cycler:

Stage	Step	Temp.	Time
Holding	Enzyme activation	95°C	10 min
Cycling (16 cycles)	Denature	95°C	15 sec
	Anneal/extend	60°C	4 min
Holding	Enzyme deactivation	99°C	10 min

STOPPING POINT Place plate on ice until you are ready to proceed, or store it at -20°C.

Note: The total sample volume of preamplified cDNA is 26.5 µL.

Perform the real-time PCR run

Dilute the preamplified samples and analyze them using a real-time PCR instrument as follows. For a detailed information on setting up and running experiments, refer to the documentation for your Applied Biosystems® Real-Time PCR System.

1. Prepare a sufficient volume of a 1:10 dilution of each preamplified product using 1X TE buffer (pH 8.0).

   IMPORTANT! You do not need to dilute the entire sample.

2. In a reaction tube of sufficient size, prepare a PCR reaction mix for each diluted sample. Scale the volumes accordingly to match the number of replicates that you intend to run.

Component	Vol. per rxn.	
	96-well plate	384-well plate
TaqMan® Gene Expression Master Mix (2X)	25.0 µL	10.0 µL
Preamplified product (diluted 1:10 with 1X TE buffer, pH 8.0)	10.0 µL	4.0 µL
TaqMan® Gene Expression Assay (20X)	2.5 µL	1.0 µL
Nuclease-free water	12.5 µL	5.0 µL
Total volume (per reaction)	**50.0 µL**	**20.0 µL**

Note: Keep the reaction plates on ice until you are ready to load them into the real-time PCR instrument.

3. For each sample, transfer 20 µL (384-well plates) or 50 µL (96-well plates) of PCR master mix to the appropriate wells of a MicroAmp® Optical Reaction Plate.

4. Seal the reaction plate and load it into your real-time PCR instrument.

5. Program your Applied Biosystems® Real-Time PCR System with the experiment details, sample/assay information, plate configuration, and the following PCR method.

Stage	Step	Temp.	Time
Holding	UDG Incubation	50°C	2 min
Holding	Enzyme Activiation	95°C	10 min
Cycling (40 cycles)	Denature	95°C	5 sec
	Anneal/Extend	60°C	1 min

Note: For specific instructions on setting up and running an experiment, refer to the user documentation for your Applied Biosystems® Real-Time PCR System.

6. Start the run.

7. When the run is complete, analyze the real-time data using the appropriate analysis module of your instrument software as explained in the user documentation.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

For support visit **lifetechnologies.com/support** or email **techsupport@lifetech.com**

lifetechnologies.com

2 October 2014