AccuLift Rapid RNA Extraction Kit

Contents

About This Document

This protocol describes how to prepare RNA for RT-PCR amplification genotyping experiments using the AccuLift™ Rapid RNA Extraction Kit.

IMPORTANT Before using this kit, read and understand the detailed instructions and safety guidelines in this document. For complete safety information, see Appendix A .

Safety Alert Conventions

Fluidigm documentation uses specific conventions for presenting information that may require your attention. Refer to the following safety alert conventions.

Safety Alerts for Chemicals

For hazards associated with chemicals, this document follows the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and uses indicators that include a pictogram and a signal word that indicates the severity level:

Indicator	Description
	Pictogram (see example) consisting of a symbol on a white background within a red diamond-shaped frame. Refer to the individual safety data sheet (SDS) for the applicable pictograms and hazards pertaining to the chemicals being used.
DANGER	Signal word that indicates more severe hazards.
WARNING	Signal word that indicates less severe hazards.

Safety Data Sheets

Read and understand the SDSs before handling chemicals. To obtain the SDS for this kit, contact your Fluidigm representative. To obtain SDSs for other chemicals ordered from Fluidigm, either alone or as part of this system, go to fluidigm.com/sds and search for the SDS using either the product name or the part number.

Some chemicals referred to in this user guide may not have been provided with your system. Obtain the SDSs for chemicals provided by other manufacturers from those manufacturers.

Introduction

The AccuLift Rapid RNA Extraction Kit is a fast, simple, and inexpensive method for preparing RNA for RT-PCR amplification. The AccuLift Rapid RNA Extraction Kit is a single-tube system that requires only vortex mixing to lyse the cells and prepare the RNA for cDNA synthesis. This allows easy processing of one to hundreds of samples in minutes, with no sample loss and without the use of toxic organic solvents. The AccuLift Rapid RNA Extraction Solution works with adherent and suspension cells and has been tested on human, mouse, rat, and *E. coli* cell cultures. The RNA extracts have been used successfully for both endpoint and real-time RT-PCR. An optional DNase I treatment may improve amplification performance by removing cellular DNA that may cause false-positive results.

Product Specifications

Storage: Store the kit at –20 °C in a freezer without a defrost cycle.

Storage buffer: RNase-Free DNase I is supplied in a 50% glycerol solution containing 10 mM Tris-HCl (pH 7.5), 10 mM $CaCl_2$, and 10 mM $MgCl_2$.

Unit definition: One molecular biology unit (MBU) of RNase-Free DNase I converts 1 µg of pUC19 DNA into oligodeoxynucleotides in 10 min at 37 °C.

Quality control: The AccuLift Rapid RNA Extraction Kit is function-tested by assaying for a PCR product from cDNA produced from RNA extracted from HeLa cells.

Contaminating activity assays: All components of the AccuLift Rapid RNA Extraction Kit are free of detectable RNase activity, and all of the components except DNase I are free of detectable exo- and endonuclease activities.

Materials

Required Kit

Product Name	Source	Cat. No.	Storage
AccuLift™ Rapid RNA Extraction Kit for LCM, 50 mL (500 extractions)	Fluidigm	10006	–20 °C
• DNase I RNase-Free (1 U/µL), 2.5 mL (PN 10031) • AccuLift RNase Inhibitor, 1.25 mL (PN 10034) • Stop Solution, 2 mL (PN 10033) • 10X DNase I Reaction Buffer, 5.5 mL (PN 10032) • AccuLift Rapid RNA Extraction Solution, 50 mL (PN 10030)			

Optional Reagent

Product Name	Source	Part Number
PBS Buffer	Major laboratory supplier (MLS)	—

Required Consumable

Product	Source	Part Number
1.5 mL microtubes	MLS	—

Required Equipment

Product	Source	Part Number
Centrifuge for microtubes	MLS	—
Pipettes and appropriate low-retention, filtered tips	MLS	—
Vortexer	MLS	—

* Recommended: Rainin® pipettes

Suggested Equipment

Product	Source	Part Number
Heating block for 1.5 mL tubes	MLS	—
Standard thermal cycler	MLS	—

* Recommended: Rainin pipettes

Best Practices

IMPORTANT Read and understand the safety information in Appendix A .
For the overall success of the protocol, we recommend the following best practices.

- Perform RNA extractions in an RNase-free work environment.
  - Always wear gloves when handling samples containing RNA and kit components.
  - Do not pick up any kit component with an ungloved hand.
  - Keep all kit components tightly sealed when not in use.
  - Keep all tubes containing RNA tightly sealed during the incubation steps.
- The extraction protocol is scalable. The reagent volume can be adjusted according to the number of cells being lysed.
- Lysates should be kept on ice when in use and when possible, used directly in cDNA synthesis reactions. Unused lysates should be stored frozen at –70 °C.

Tissue Samples via Laser Capture Microdissection

1. Retrieve AccuLift Rapid RNA Extraction Solution (10030) from –20 °C, thaw, and keep on ice.
2. Vortex reagents for 20 sec, and then briefly centrifuge them before use.
3. Microdissect your cell population of interest from a frozen tissue section.

4 Immediately after microdissection, place the AccuLift LCM GeckoGrip™ Cap in a 0.5 mL tube with 25 µL of RNA Extraction Solution.

5 Place the samples on dry ice for 1 min, and then remove all samples from dry ice. Let all samples thaw (~2 min).

6 Briefly vortex the sample to extract the microdissected cells from the cap surface.

7 Spin AccuLift LCM GeckoGrip™ Cap and 0.5 mL tube at 800 x *g* for 2 min to collect all of the cell extract. Proceed to step 10.

Optional Protocol

1 Immediately after microdissection, pipet 25 µL of RNA Extraction Solution directly on the microdissected cells on the LCM Cap.

2 Place the cap onto a 1.5 mL microcentrifuge tube to rest on dry ice while remaining samples are microdissected.

NOTE Do not place cap into a 0.6 mL tube. While this tube fits the cap, snapping the cap onto the tube will disrupt the RNA Extraction Solution. Placing the cap loosely on a larger tube allows the sample to remain over dry ice without disturbing the RNA extraction buffer bead over microdissected cells.

3 Once all samples have been microdissected and 25 µL of RNA Extraction Solution added to each and refrozen over dry ice (~1 min), remove all samples from dry ice. Let all samples thaw (~2 min).

4 For each sample, pipet entire 25 µL of RNA Extraction Solution up and down until all cells are removed from LCM Cap surface (1 min/sample).

5 For each sample, remove 25 µL of RNA Extraction Solution and proceed through AccuLift Cleanup and Concentrator Kit (PN 10143).

Optional Next Step

Use AccuLift Ultra-Sensitive RNA Amplification Kit (PN 10146) for LCM to generate total cDNA directly with the following protocol:

1 Take 2 µL of each sample and add to 1 µL AccuLift T7-Oligo(dT) Primer A.

2 Begin at Step 2 of AccuLift Ultra-Sensitive RNA Amplification Kit.

3 For each sample, add 25 µL of sample to 50 µL of RNA binding buffer, and mix by pipetting.

4 Add 75 µL of 95–100% ethanol to each sample and mix gently.

5 Begin at Step 3 of AccuLift Cleanup and Concentrator Kit Protocol (PN 10143).

Following isolation of purified RNA, we recommend using AccuLift First Strand cDNA Synthesis Kit (PN 1014X) with the primers of your choice for cDNA synthesis.

NOTE Following AccuLift RNA Spin Column Micro Prep Kit RNA isolation, both AccuLift First Strand cDNA Synthesis Kit and AccuLift Ultra-Sensitive RNA Amplification Kit for LCM can be used with the same sample for direct comparison if desired. Use 20 of 25 µL per sample for First Strand, and 2 of 25 µL per sample for the Ultra-Sensitive Kit.

Prepare Adherent or Suspension Cultured Cells (10^3 to 10^6 cells)

1. Retrieve AccuLift Rapid RNA Extraction Solution (PN 10030) from –20 °C, thaw, and keep on ice.
2. Vortex reagents for 20 sec, and then briefly centrifuge them before use.
3. Harvest the cells using a method appropriate to the cell type and growth vessel. If trypsin is used for adherent cells harvest, it should be inactivated.
4. Count the cells.
5. (Optional) Wash cells with PBS or similar buffer to remove serum and media components.
6. Pellet the cells by centrifugation at 1,000 x *g* at 4 °C for 5 min. Carefully aspirate the supernatant.
7. Add 100 µL of ice-cold AccuLift Rapid RNA Extraction Solution to the cells and vortex mix for 1 full min.

   NOTE The RNA can be used directly in a cDNA synthesis reaction or stored frozen at –70 °C.
8. (Optional) Remove the DNA with a DNase I treatment.

Optional DNase I Treatment

An optional DNase I treatment may improve amplification performance by removing cellular DNA that may cause false-positive results.

IMPORTANT Preheat the lysate at 65 °C for 2 min in a thermal cycler before proceeding.

1. Retrieve reagents from storage:

Required Reagent	Preparation
DNase I RNase-Free (1 U/µL), 2.5 mL (PN 10031)	Remove from –20 °C, thaw, and keep on ice.
AccuLift RNase Inhibitor, 1.25 mL (PN 10034)	
Stop Solution, 2 mL (PN 10033)	
10X DNase I Reaction Buffer, 5.5 mL (PN 10032)	

2 Add 10X DNase I Reaction Buffer, RiboGuard RNase Inhibitor, and DNase I RNase-free into the same tube as the extracted RNA. Mix the sample and incubate the tube at 37 °C for 15 min.

Component	Volume (µL)*
10X DNase I Reaction Buffer (PN 10032)	1 1
AccuLift RNase Inhibitor (PN 10034)	2.5
DNase I RNase-Free (PN 10031)	5
Extracted RNA	??
Total	**??**

3 Add 4 µL of Stop Solution to the tube, mix well, and incubate at 65 °C for 10 min.

4 Store the tubes on ice or frozen until the sample is to be used for cDNA synthesis.

PCR Amplification Recommendations for cDNA and DNA

- For cDNA synthesis, 1–10 µL of extracted RNA can be used directly with any reverse transcriptase and a standard 20-µL protocol. Up to 50% of the reaction volume can be extracted RNA.
- For standard and fast endpoint PCR cycling profiles, use 1–5 µL of cDNA.
- When using extracts in real-time RT-PCR with SYBR® Green I Dye, or other similar dye detection, DNase treatment of the extract before cDNA synthesis is recommended to remove DNA that can cause false-positive PCR results. All of the reagents required for DNase I treatment are included in the kit.
- If possible, design primers so that DNase I treatment is not needed. Primers can be designed to prevent amplification of genomic RNA by choosing sequences that flank a long intron. Short cycling times are then used so that the DNA amplicon is not produced while the shorter cDNA amplicon is efficiently produced. Primers can also be chosen that anneal to exon-exon boundaries in the cDNA. These sequences do not exist in the genomic DNA.

Appendix A: Safety

General Safety

In addition to your site-specific safety requirements, Fluidigm recommends the following general safety guidelines in all laboratory and manufacturing areas:

- Use personal protective equipment (PPE): safety glasses, fully enclosed shoes, lab coats, and gloves.
- Know the locations of all safety equipment (fire extinguishers, spill kits, eyewashes/showers, first-aid kits, safety data sheets, etc.), emergency exit locations, and emergency/injury reporting procedures.

- Do not eat, drink, or smoke in lab areas.
- Maintain clean work areas.
- Wash hands before leaving the lab.

Chemical Safety

The responsible individuals must take the necessary precautions to ensure that the surrounding workplace is safe and that instrument operators are not exposed to hazardous levels of toxic substances. When working with any chemicals, refer to the applicable safety data sheets (SDSs) provided by the manufacturer or supplier.

Disposal of Products

Used reagents should be handled and disposed of in accordance with federal, state, regional, and local laws for hazardous waste management and disposal.