AccuLift RNA Spin Column Micro Prep Kit

Contents

About This Document

This protocol describes how to use the AccuLift™ RNA Spin Column Micro Prep Kit to isolate DNA-free RNA from cell and tissue samples.

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

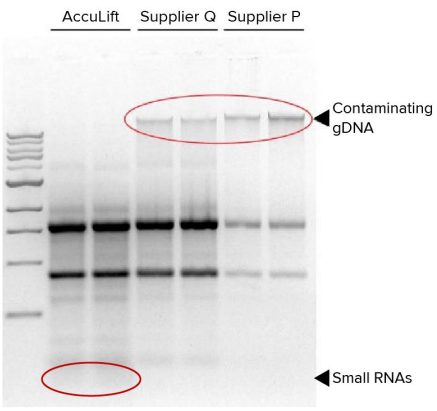
Read and understand the SDSs before handling chemicals. To obtain the SDS for this kit, contact your Fluidigm representative. To obtain SDSs for other chemicals ordered from Fluidigm, either alone or as part of this system, go to fluidigm.com/sds and search for the SDS using either the product name or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

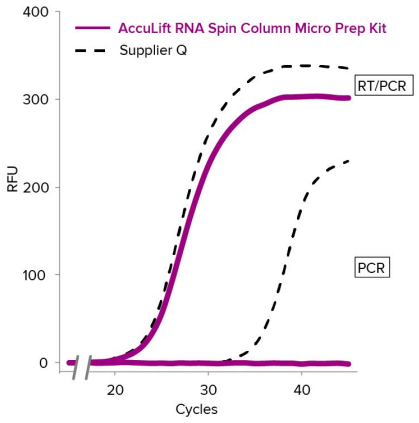
Some chemicals referred to in this user guide may not have been provided with your system. Obtain the SDSs for chemicals provided by other manufacturers from those manufacturers.

Introduction

The AccuLift RNA Spin Column Micro Prep Kit is an innovative product designed for the easy, reliable, and rapid isolation of DNA-free RNA from a wide range of cell (up to 10^6) and tissue samples (up to 5 mg). The procedure combines a unique buffer system with Clean-Spin™ column technology to yield high-quality total RNA (including small RNAs 17–200 nt) in about 10 min.

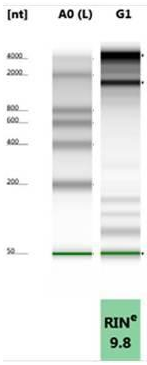
The procedure: Add the provided RNA Lysis Buffer to a sample, then purify the RNA using the AccuLift Spin Columns. The result is highly-concentrated, DNA free RNA that is suitable for subsequent RNA-based methods including RT-PCR, hybridization, and sequencing. In addition, the kit can be used for enrichment of small and large RNAs in two separate fractions.





The AccuLift RNA Spin Column Micro Prep Kit yields high-quality total RNA. High levels of genomic DNA contamination are present in the preps from Suppliers Q & P but not with the AccuLift RNA Spin Column Micro Prep Kit. Total RNA was isolated from human epithelial cells (sans DNase treatment).

RNA isolated with the AccuLift RNA Spin Column Micro Prep Kit is DNA-free. Samples isolated with Supplier Q's kit are provided for comparison. Total RNA was isolated from 10^6 human epithelial cells (with in-column DNase treatments for both kits). Each amplification curve represents an average of three independent isolation experiments.



The AccuLift RNA Spin Column Micro Prep Kit yields high-quality RNA with high RNA integrity numbers (2200 TapeStation, Agilent®).

Specifications

NOTE Some difficult-to-lyse samples may require mechanical or enzymatic homogenization. For assistance, contact your Fluidigm representative.

- **Sample sources:** Cells or tissue samples, yeast, plant, or bacteria. Compatible with DNA/RNA Shield™ and RNA*later*™.
- **Sample storage:** Samples homogenized in RNA Lysis Buffer are stable and can be stored frozen prior to purification.
- **Sample size:** Up to 10^6 cells or 5 mg tissue.
- **RNA purity:** High-quality RNA (A_{260}/A_{280} >1.8, A_{260}/A_{230} >1.8) suitable for all downstream RNA-based manipulations.
- **RNA recovery:** Up to 10 µg RNA can be eluted into ≥6 µL RNase-free water, allowing for a highly concentrated sample.
- **RNA storage:** RNA is eluted with RNase-free water and can be stored frozen. RNase inhibitors can be included for prolonged storage.

Materials

Required Kit from Fluidigm

IMPORTANT Store reagents as soon as they are received, according to manufacturer's storage recommendations.

Product Name	Source	Catalog Number	Storage
AccuLift RNA Spin Column Micro Prep Kit, (50 Reactions) Kit contains: • RNA Lysis Buffer, 50 mL (PN 10077) • RNA Prep Buffer, 25 mL (PN 10070) • RNA Wash Buffer (concentrate)*, 24 mL (PN 10078) • DNase/RNase-Free Water, 4 mL (PN 10074) • DNase I Set† (lyophilized) (PN 10072) • DNA Digestion Buffer (PN 10073) • AccuLift IC Columns (PN 10075) • Collection Tubes (PN 10076)	Fluidigm	10010	Room temperature

* Before use, prepare the RNA Wash Buffer.

Required Equipment

Product	Source	Part Number
Microcentrifuge	MLS	—
Pipettes* and appropriate low-retention, filtered tips	MLS	—

* Recommended: Rainin® pipettes

Prepare Reagents

Before isolating RNA, prepare the following reagents:

RNA Wash Buffer

Before starting, add 96 mL 100% ethanol (104 mL 95% ethanol) to the 24 mL RNA Wash Buffer concentrate.

DNase I

Reconstitute the lyophilized DNase I as indicated on the vial prior to use and store aliquots at –20°C.

Isolate RNA

Ensure the RNA isolation procedure is performed in an RNase-free environment.

RNA isolation consists of three steps:

1 Lyse or homogenize samples

2 Clear samples

3 Purify RNA

NOTE All steps should be performed at room temperature (20–30 °C).

Sample Lysis/Homogenization

Samples homogenized in RNA Lysis Buffer can be stored frozen for processing at a later time.

	RNA Lysis Buffer	
Sample Type	**100 µL**	**300 µL**
Cells	Up to 10%	Up to 10%
Tissues	—	Up to 5 mg

Sample Type	Protocol
Adherent cells	Lyse cells directly in the culture container by removing liquid medium and adding RNA Lysis Buffer directly to the monolayer.
Cells in suspension	1 Pellet cells (≤500 x *g*), remove the supernatant completely, and then resuspend the cell pellet in RNA Lysis Buffer. 2 Vortex briefly.
Tissue samples via laser capture microdissection	1 Microdissect the cell population of interest from a frozen tissue section. 2 Immediately post microdissection, pipet 50 µL of RNA Lysis buffer directly into a 0.5 mL microcentrifuge tube and snap the cap with microdissected samples into the microcentrifuge tube. Invert and incubate the tube at room temperature for 1 min. 3 Briefly vortex the sample to extract the microdissected cells from the cap surface. 4 Centrifuge AccuLift LCM GeckoGrip™ Caps and 0.5 mL tube at 800 x *g* for 2 min to collect all of the cell extract. • **Optional protocol for Step 4:** For each sample, remove the cap and pipet the entire 50 µL of RNA Lysis Buffer up and down on the cap surface to remove any cells that remain on the AccuLift LCM GeckoGrip Caps surface. Replace the AccuLift LCM GeckoGrip Caps on 0.5 mL microcentrifuge tube and centrifuge at 800 x *g* for 2 min to collect all of the cell extract. 5 Remove AccuLift LCM GeckoGrip Caps and discard. Save the lysed cell and proceed to Sample Clearing Step. Alternatively, cell extract can be frozen at –80 °C until later downstream analysis.

Sample Type	Protocol
Tissue and tough-to-lyse samples	Fresh or frozen tissue (animal, plant, insect, yeast or bacteria) can be mechanically homogenized directly in the RNA Lysis Buffer. **NOTE** Alternatively, tough-to-lyse tissue samples can be Proteinase K treated.

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Sample Type	Protocol
Liquids/reaction clean-up	DNase-treated RNA, labeling, and *in vitro* transcription reactions can be processed directly by adding 4 volumes of RNA Lysis Buffer to each volume of sample (4:1) then mixing well.
Samples in DNA/RNA Shield	**1** Bring samples homogenized and stored in DNA/RNA Shield to room temperature (20–30 °C). **2** Add 1 volume RNA Lysis Buffer (1:1), mix and proceed with Sample Clearing step. **NOTE** Samples in DNA/RNA Shield can be Proteinase K treated. Use the DNA/RNA Shield for safe sample storage and transport at ambient temperatures
Samples in RNA*later*	To process cells or liquids in RNA*later* (without reagent removal): **1** Add 1 volume of RNase-free water or PBS to the sample (1:1). **2** Add 4 volumes of RNA Lysis Buffer (4:1) and mix. **NOTE** Alternatively, remove the RNA*later*, then proceed with Sample Lysis/Homogenization according to the sample type.

* ZR BashingBead™ lysis tubes are available separately (Cat. No.S6002, S6003).

Sample Clearing

Sample clearing is recommended for cells and tissue (animal/plant) but can be omitted for cell-free liquids and low-input samples (≤10^5 cells).

1 For particulate removal, centrifuge lysates at ≥12,000 x *g* for 1 min.

2 Transfer the supernatant into an RNase-free tube (not provided).

RNA Purification

All centrifugation steps should be performed at 10,000–16,000 *x g.*

Unit definition: One unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A_{260} units/min/mL of reaction mixture at 25 °C.

1 Add 1 volume ethanol (95–100%) to the sample in RNA Lysis Buffer (1:1). Mix well.

2 Transfer the mixture to an AccuLift IC Column in a collection tube and centrifuge for 30 sec. Discard the flow-through.

NOTE To process samples >700 µL, AccuLift IC Columns may be reloaded.

3 (Optional) Perform an in-column DNase I treatment to remove trace DNA.

a Prewash the column with 400 µL RNA Wash Buffer. Centrifuge for 30 sec. Discard the flow-through.

b For each sample to be treated, prepare DNase I reaction mix in an RNase-free tube (not provided). Mix well by gentle inversion:

Component	Volume (µL)
DNase I	5
DNA Digestion Buffer	35

c Add 40 µL DNase I reaction mix directly to the column matrix. Incubate at room temperature (20–30 °C) for 15 min.

4 Add 400 µL RNA Prep Buffer to the column and centrifuge for 30 sec. Discard the flow-through.

5 Add 700 µL RNA Wash Buffer to the column and centrifuge for 30 sec. Discard the flow-through.

6 Add 400 µL RNA Wash Buffer and centrifuge the column for 2 min to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube (not provided).

7 Add 15 µL DNase/RNase-Free Water directly to the column matrix and centrifuge for 30 sec.

NOTE Alternatively, for highly concentrated RNA use ≥6 µL elution.

STOPPING POINT The eluted RNA can be used immediately or stored at –70°C.

Purification of Small and Large RNAs into Separate Fractions

This procedure is compatible with animal cell inputs (up to 10^6) or previously isolated RNA only.

All centrifugation steps should be performed between 10,000–16,000 x *g*. This protocol requires two columns (per prep).

1 Mix an equal volume of RNA Lysis Buffer and ethanol (95–100%).

Example: Mix 50 µL buffer and 50 µL ethanol.

2 Add 2 volumes of the buffer/ethanol to an RNA sample [adjust the sample volume to 50 µL (minimum)] or 300 µL buffer/ethanol to a cell pellet and mix.

Example: Mix 100 µL buffer/ethanol and 50 µL sample.

3 Transfer the mixture to the AccuLift IC Column and centrifuge for 30 sec.

IMPORTANT **Save the flow-through!**

NOTE AccuLift Spin Columns may be reloaded to process samples >700 µL.

4 **For Columns (RNAs >200 nt):** Continue to Step 5.
For Flow-through (RNAs 17–200 nt): Add 1 volume ethanol and mix. Transfer the mixture to a new column and centrifuge for 30 sec. Discard the flow-through.

Example: Add 150 µL ethanol to 150 µL flow-through.

5 Add 400 µL RNA Prep Buffer to the column and centrifuge for 30 sec. Discard the flow-through.

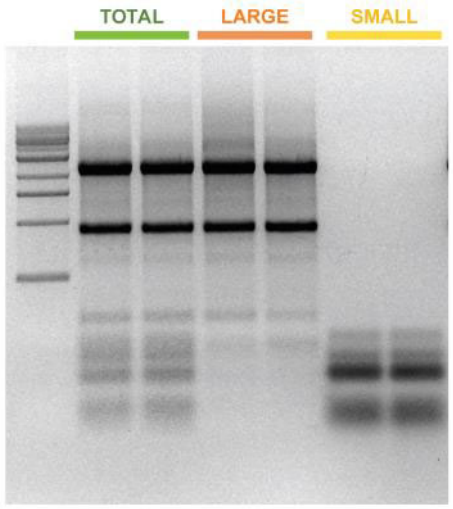
6 Add 700 µL RNA Wash Buffer to the column and centrifuge for 30 sec. Discard the flow-through.

7 Add 400 µL RNA Wash Buffer and centrifuge the column for 2 min to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube (not provided).

8 Add 15 µL DNase/RNase-Free Water directly to the column matrix, then centrifuge for 30 sec.

NOTE Alternatively, for highly concentrated RNA use ≥6 µL elution.

STOPPING POINT The eluted RNA can be used immediately or stored at –70 °C.



Total RNA (>17 nt), large (>200 nt) or small RNAs (17–200 nt) are effectively partitioned and purified with the AccuLift RNA kit.

Proteinase K Digestion

Example reaction mix

Component	Volume
Up to 5 mg solid tissue or 10^6 animal cells in DNA/RNA Shield	95 µL
2X Digestion Buffer (Zymo Cat. No. D3050-1-5 and D3050-1-20).	95 µL

Proteinase K* (Zymo Cat. No. D3001-2-5 and D3001-2-20).	≥6 U

* One unit of enzyme will hydrolyze urea-denatured hemoglobin to produce 1.0 µmole of tyrosine per min at pH 7.5 at 37 °C.

1. Prepare a Proteinase K reaction mix (see example above, scale up as necessary).
2. Incubate at 55 °C for 30 min (for example, for pelleted white blood cells) or 1—3 hours (for solid tissue).
3. Add 1 volume RNA Lysis Buffer and go back to Sample Clearing.

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.