AccuLift RNA Cleanup and Concentrator Kit

Contents

About This Document

This protocol describes how to use the AccuLift™ RNA Cleanup and Concentrator Kit to recover ultrapure RNA [≥17 nucleotide (nt)] from enzymatic reactions, aqueous phase (following TRIzol™ extraction, Thermo Fisher Scientific), and other sources.

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.

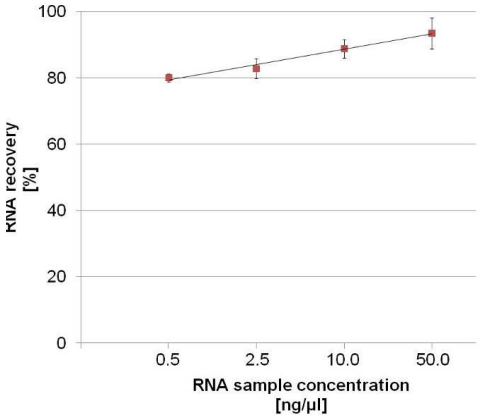
Some chemicals referred to in this user guide may not have been provided with your system. Obtain the SDSs for chemicals provided by other manufacturers from those manufacturers.

Introduction

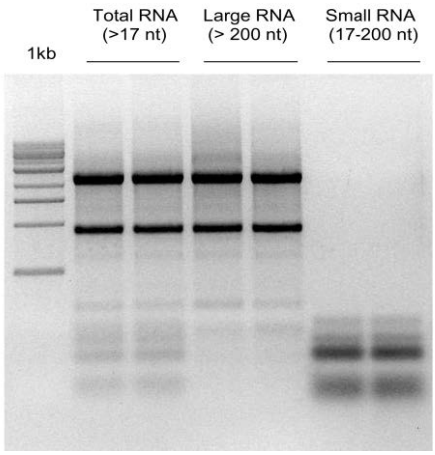
The AccuLift RNA Cleanup and Concentrator Kit provides a simple and reliable method for the rapid preparation of up to 10 µg of high-quality RT-PCR-ready, DNA-free RNA. This procedure is based on the use of a unique single-buffer system and CleanSpin™ column technology that allows for selective recovery of total RNA (>17 nt), large RNAs (>200 nt), and/or small RNAs (17–200 nt).

The procedure: Add binding buffer and ethanol to your sample, then bind, wash, and elute ultrapure RNA. The RNA can be eluted from the AccuLift IC Columns in as little as 6 µL of RNase-free water. The highly concentrated, purified RNA is suitable for all subsequent analyses and molecular manipulations.

The entire procedure typically takes about 5 min.



Concentration of diluted RNA samples. RNA was eluted with 20 µL RNase-free water (n = 3, total input = 1 µg RNA).



Purification of small and large RNAs into separate fractions. The AccuLift RNA Cleanup and Concentrator Kit allows for purification of total RNA (>17 nt), large RNAs (>200 nt), and/or small RNAs (17–200 nt).

Specifications

- **Sample sources:** DNase I treated RNA, *in vitro* transcription products, the aqueous phase following TRIzol/chloroform, or similar extraction.

  NOTE Extraction is compatible with: TRIzol, TRI Reagent®, RNAzol®, QIAzol®, TriPure™, TRISure™ and other acid-guanidinium-phenol reagents.

- **RNA size limits:** From 17 nt to ~23 kb
- **RNA purity:** High-quality RNA (A_{260}/A_{280} >1.8, A_{260}/A_{230} >1.8) suitable for reverse transcription, microarray, sequencing etc.
- **RNA recovery:** Up to 10 µg RNA can be eluted into as little as 6 µL RNase-free water, allowing for a highly concentrated sample.
- **RNA storage:** RNA is eluted with RNase-free water and can be stored at ≤-70 °C. The addition of RNase inhibitors is optional but highly recommended for prolonged storage.

Materials

Required Kit from Fluidigm

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

NOTE Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure that they provide the highest performance and reliability.

Product Name	Source	Part Number	Storage
AccuLift RNA Cleanup and Concentrator Kit (50 Reactions) Kit contains: • RNA Binding Buffer, 25 mL (PN 10069) • RNA Prep Buffer, 25 mL (PN 10070) • RNA Wash Buffer (concentrate)*, 12 mL (PN 10071) • DNase I Set† (lyophilized) (PN 10072) • DNA Digestion Buffer, 4 mL (PN 10073) • DNase/RNase-Free Water, 4 mL (PN 10074) • AccuLift IC Columns (PN 10075) • Collection Tubes (PN 10076)	Fluidigm	10012	Room temperature

* Before use, prepare the RNA Wash Buffer.

† Prior to use, reconstitute the lyophilized DNase I.

Required Reagents from Other Suppliers

Product	Source	Part Number
Ethanol, 95%	Major laboratory supplier (MLS)	—

* Recommended: Rainin® pipettes

Suggested Reagents from Other Suppliers

Product	Source	Part Number
TE Buffer		—

Required Consumables from Other Suppliers

Product	Source	Part Number
RNase-free tube	MLS	—

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 48 mL 100% ethanol (52 mL 95% ethanol) to the 12 mL RNA Wash Buffer concentrate.

DNase I

Prior to use, reconstitute the lyophilized DNase I as indicated on the vial and store aliquots at –20 °C.

DNase I Treatment

There are two methods of performing the DNase I digestion: before the cleanup and during the cleanup (in-column). Choose an appropriate method for your application below:

Before Cleanup

The DNase digestion procedure can be performed using the DNase I Set. Prior to use, reconstitute the lyophilized DNase I.

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 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)
RNA sample (≤10 µg), volume adjusted with water or TE buffer	40
DNase I	5
DNA Digestion Buffer	5
Total Volume	**50**

2 Incubate at room temperature (20–30 °C) for 15 min. Then start with RNA Purification.

In-Column

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

1 Following the RNA binding step (RNA Purification), prewash the column with 400 µL RNA Wash Buffer. Centrifuge for 30 sec. Discard the flow-through.

2 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

3 Add 40 µL reaction mix directly to the column matrix. Incubate at room temperature (20–30 °C) for 15 min. Then continue with RNA Purification.

RNA Purification from Aqueous Phase after TRIzol Extraction

1 Following TRIzol/chloroform or similar extraction, carefully transfer the upper aqueous phase into an RNase-free tube (not provided).

NOTE Extraction is compatible with: TRIzol, TRI Reagent, RNAzol, QIAzol, TriPure, TRISure and other acid-guanidinium-phenol reagents.

2 For each volume of the aqueous phase (as measured or estimated), add 1 volume ethanol (95–100%) and mix.

3 Continue with RNA Purification.

RNA Purification

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

RNA species ≥17 nt will be recovered.

For DNA-free RNA, perform DNase I treatment prior to or during the cleanup protocol.

1 Add 2 volumes RNA Binding Buffer to each sample and mix.

NOTE Adjust the sample volume to 50 µL (minimum).

Example: Mix 100 µL buffer and 50 µL sample.

2 Add an equal volume of ethanol (95–100%) and mix.

Example: Add 150 µL ethanol.

3 Transfer the sample to the AccuLift IC Columns in a collection tube and centrifuge for 30 sec. Discard the flow-through.

NOTE To process samples >800 µL, AccuLift IC Columns may be reloaded.

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 to the column and centrifuge 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

All centrifugation steps should be performed at 10,000–16,000 x *g*. This protocol requires 2\ columns (per prep).

1 Prepare adjusted RNA Binding Buffer (as needed). 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 adjusted buffer to the sample and mix.

Example: Mix 100 µL adjusted buffer and 50 µL sample.

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

IMPORTANT **Save the flow-through!**

- RNAs >200 nt are retained in the column: Skip to Step 5.
- RNAs 17–200 nt are in the **flow-through**: Continue to Step 4.

4 For flow-through only:

a Add 1 volume ethanol and mix.

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

b Transfer the mixture to a new column and centrifuge for 30 sec. Discard the 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.

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.