FLUIDIGM®

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PROTOCOL

AccuLift Ultra-Sensitive RNA Amplification Kit

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About This Document

This protocol describes how to use the AccuLift™ Ultra-sensitive RNA Amplification Kit to amplify RNA.

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

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

Quantitative reverse transcription-PCR (qRT-PCR) for gene expression studies from very small samples can be difficult for a number of reasons, including the following:

- Very few qRT-PCRs possible due to sample size
- Lack of sensitivity, especially of low-abundance transcripts
- Difficulty purifying minute amounts of total RNA
- Need to collect samples frequently
- Difficulty in archiving samples

An AccuLift Ultra-sensitive RNA Amplification Kit reaction amplifies, in a linear manner, the mRNA [poly(A) RNA] directly from the lysates of 1–1,000 cells, without the need for isolating total cellular RNA. The amplified RNA is converted to cDNA that can be diluted and used for qPCR. Thus, an AccuLift Ultra-sensitive RNA Amplification Kit reaction eliminates the difficulty and uncertainty of purifying total RNA from minute samples while amplifying the mRNA for significantly improved qRT-PCR results.

Workflow Overview

The one-day reaction involves:

Part A: Cell Lysis—The harvested cells are lysed using the AccuLift Rapid RNA Extraction (PN 10006) Kit and AccuLift RNA Clean and Concentrator Kit (PN 10012) or the AccuLift RNA Spin Column Micro Prep Kit (PN 10010).

Part B: Round-One, First-Strand cDNA Synthesis—The Poly(A) RNA in the cell lysate is reverse-transcribed into first-strand cDNA. The reaction is primed by an oligo(dT) primer containing a phage T7 RNA Polymerase promoter sequence at its 5′ end.

Part C: Round-One, Second-Strand cDNA Synthesis—The single-strand cDNA produced in Step 2 is converted to double-strand cDNA that contains a T7 transcription promoter.

Part D: *In Vitro* Transcription—High yields of amplified RNA are produced in an *in vitro* transcription reaction from the double-stranded cDNA template produced in Step 3.

Part E: RNA Purification—The amplified RNA is purified by spin-column chromatography (supplied by the user).

Part F: Round-Two, First-Strand cDNA Synthesis—The purified RNA is reverse-transcribed into first-strand cDNA. The reaction is primed using random-sequence hexamer primers. The cDNA produced can be diluted for qPCR using a qPCR system of the user’s choosing.

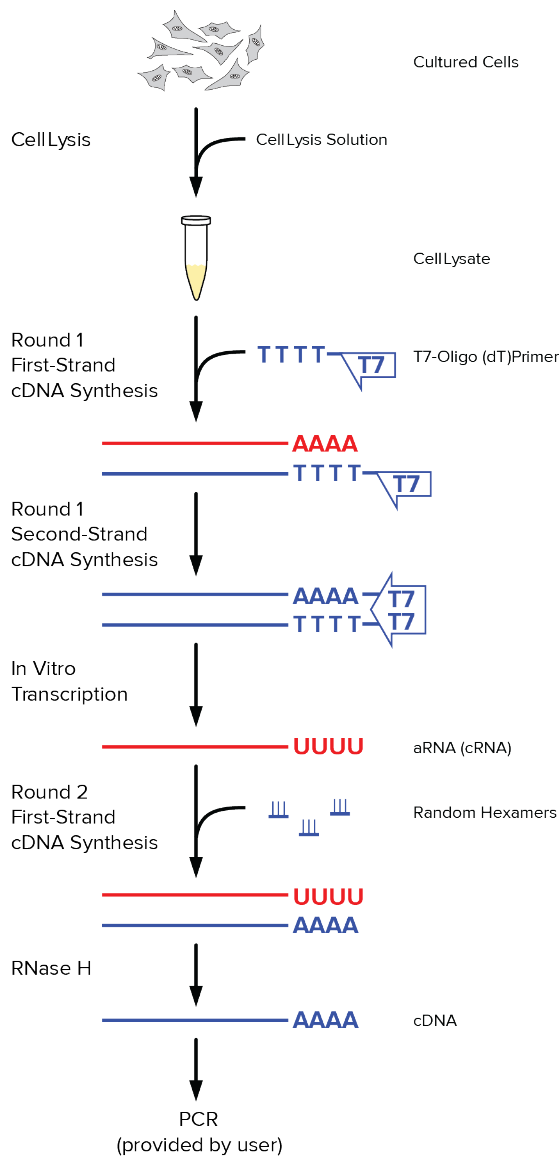

Figure 1. Overview of the AccuLift Ultra-sensitive RNA Amplification Kit process

Product Specifications

Quality Control

The AccuLift Ultra-sensitive RNA Amplification Kit is function-tested using the normal rat kidney (NRK) Total RNA Control and Control PCR Primers [specific for the low-abundance Porphobilinogen Deaminase (PBGD)] mRNA provided in the kit. One microliter of cDNA produced from 50 pg of NRK Total RNA Control yields a CT value of <30 cycles in PCR with the cycling conditions described in Appendix C. Melting curve analysis demonstrates a single sharp peak with a Tm ~88 °C.

Materials

Required Kit from Fluidigm

IMPORTANT Upon receipt of this kit, remove the tube containing the NRK Total RNA Control and store it at –70 °C to –80 °C. Store the remainder of the kit at –20 °C.

Product Name	Source	Cat. No.	Cap Color
AccuLift Ultra-sensitive RNA Amplification Kit (10 Reactions)	Fluidigm	10007	
• T7-Oligo(dT) Primer A, 20 µL (PN 10056) • RT PreMix, 50 µL (PN 10059) • DNA Polymerase PreMix, 90 µL (PN 10055) • DNA Polymerase, 10 µL (PN 10049) • cDNA Finishing Solution, 20 µL (PN 10050)			Red
• Random Primers, 40 µL (PN 10057) • RNase H, 10 µL (PN 10048) • MMLV-RT, 10 µL (PN 100047) • IVT PreMix A, 280 µL (PN 10054)			Blue
• T7 RNA Polymerase, 40 µL (PN 10045) • 10X Transcription Reaction Buffer, 40 µL (PN 10058) • RNase-Free DNase I (1 U/µL), 60 µL (PN 10046)			Green
• Forward Control PCR Primer, 10 µL (PN 10060) • Reverse Control PCR Primer (12.5 µM), 10 µL (PN 10061)			Yellow
• DTT (100 mM), 50 µL (PN 10052) • Nuclease-Free Water, Sterile, 1.0 mL (PN 10062) • Poly(I), 15 µL (PN 10053) • AccuLift Total RNA Control (50 ng/µL), 10 µL (PN 10088)			Clear

Required Reagent from Other Suppliers

Product Name	Source	Part Number
RNase-Free Water	Major laboratory supplier (MLS)	—

Optional Reagent

Product Name	Source	Part Number
Phosphate-Buffered Saline (PBS)	MLS	—

Required Consumables

Product	Source	Part Number
1.5 mL microtubes	MLS	—
RNA Purification Columns*		

* Recommended: RNA Clean and Concentrator™-5 Kit (Fluidigm) or RNeasy® MinElute® Cleanup Kit (Qiagen®)

Required Equipment

Product	Source	Part Number
Microcentrifuge	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 or water bath	MLS	—

Best Practices

IMPORTANT Read and understand the safety information in Appendix D.

For the overall success of the protocol, we recommend the following best practices.

Maintaining an RNase-Free Environment

Ribonuclease contamination is a significant concern for those performing RNA amplification and RT-PCR. Creating an RNase-free work environment and maintaining RNase-free solutions is critical for performing successful RNA amplification reactions. Therefore, we strongly recommend that the user:

- Use RNase-free (for example, autoclaved) tubes and pipette tips.
- Always wear gloves when handling samples containing RNA. Change gloves frequently, especially after touching potential sources of RNase contamination such as door knobs, pens, pencils, and human skin.
- Always wear gloves when handling 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.

Familiarizing Yourself with the Kit Components

The AccuLift Ultra-sensitive RNA Amplification Kit includes many components. Before starting, please read the procedure carefully and familiarize yourself with each kit component and in which step of the procedure it is used. Be sure to wear gloves when handling the kit components.

Preparing LCM samples and Cells

A kit reaction is optimized for use with 1–1,000 cells per reaction. Cells obtained by laser-capture microdissection (LCM), cultured cells, flow-sorting methods, or biopsy and tissue samples can all be used successfully. Tissue samples must be dissociated for optimal results. Detach adherent cultured cells using standard laboratory procedure. If using proteolytic enzymes, for example trypsin, add additional serum and collect the cells by centrifugation. Resuspend the cells in serum and count (or estimate) the desired number of cells and place them into a sterile 0.5 mL microcentrifuge tube. Pellet the cells and carefully aspirate off the serum. Proceed with Part A or, if desired, wash the cells with ice-cold phosphate-buffered saline (PBS). Centrifuge the washed cells and carefully aspirate the PBS. Place the cells on ice and continue with cell lysis (Part A). We do not recommend using formalin-fixed paraffin-embedded (FFPE) samples.

Performing a Control Reaction

We strongly recommend that those users who are not experienced with the kit perform a control reaction (Appendices B and C) prior to committing a valuable sample. Purified NRK Total RNA Control and forward and reverse PCR primers for detecting a low-abundance transcript (PBGD) are provided.

Performing Multiple Reactions

Very small volumes of some kit components are required for each reaction. We recommend that you prepare master mixes of reaction components when performing multiple reactions simultaneously.

Choosing Reverse Transcriptase

AccuLift Reverse Transcriptase is provided in the kit. However, other Reverse Transcriptase-type enzymes, provided by the user, such as SuperScript™ II or SuperScript III (Thermo

Fisher Scientific™) can be used if desired. Modifications to the protocol for use of either of these reverse transcriptase enzymes are noted in the procedure.

The *In Vitro* Transcription Reaction

Assemble the *in vitro* transcription reaction (Part D) at room temperature. Do not exceed the 4-hr *in vitro* transcription reaction time indicated in the procedure.

No Reverse Transcriptase Control

The AccuLift Ultra-sensitive RNA Amplification Kit reaction includes a DNase treatment at Part D, Step 5 that will significantly reduce, but not completely eliminate, the cellular genomic DNA in the sample. Therefore, we recommend that a “no reverse transcriptase” (No-RT) control reaction be run for each sample. The kit includes sufficient reagents to run a No-RT control reaction for each reaction. It may not be necessary to perform the No-RT control if using PCR primers that span exon-exon junctions in the transcript(s) of interest.

qPCR and Primer Selection

The cDNA produced by the kit is ready to be diluted (see Appendix A) for qPCR. qPCR is performed with the user’s choice of qPCR system.

Since the first-strand cDNA reaction is done using an oligo(dT) primer, we recommend selecting PCR primers near the 3′ end of the mRNA.

Stopping Points

Optional stopping points are noted following the Round One, Second-Strand cDNA Synthesis step (Part C) and after purification of the amplified RNA (Part E).

AccuLift Ultra-sensitive RNA Amplification Kit Procedure

Read through the AccuLift Ultra-sensitive RNA Amplification Kit procedure carefully before beginning. We recommend that those users who are not experienced with the kit perform a control amplification reaction (Appendices B and C) prior to committing a valuable sample. NRK Total RNA Control and PCR primers are provided with the kit.

Part A: Cell Lysis

Recommended:

AccuLift Rapid RNA Extraction Kit or the AccuLift RNA Spin Column Micro Prep Kit

Part B: Round-One, First-Strand cDNA Synthesis

Choice of Reverse Transcriptase: AccuLift Reverse Transcriptase is provided in the kit. However, other MMLV Reverse Transcriptase-based enzymes, provided by the user, such

as SuperScript II or SuperScript III (Thermo Fisher Scientific) can be used. Modifications to the procedure for use of either of the SuperScript enzymes are noted in the procedure.

Required in Part B:

Component Name	Tube Label	Cap Color
T7-Oligo(dT) Primer	T7-Oligo(dT) Primer	Red
Reverse Transcription PreMix	RT PreMix	
RiboGuard™ RNase Inhibitor	RiboGuard RNase Inhibitor	
MMLV Reverse Transcriptase	MMLV-RT	
Dithiothreitol	DTT	Clear
Nuclease-Free Water	Nuclease-Free Water, Sterile	

Incubation temperatures performed in Part B: 65 °C and 37 °C.

1. Anneal the T7-Oligo(dT) Primer to the sample.

Component	Volume (µL)
Cell lysate	3
T7-Oligo(dT) Primer	1
Total reaction volume	**4**

2. Incubate at 65 °C for 5 min in a water bath or thermocycler.

3. Chill on ice for 1 min. Centrifuge briefly in a microcentrifuge.

4. Perform first-strand cDNA synthesis:

NOTE
- If a No-RT control reaction is performed, substitute Nuclease- Free Water for the Reverse Transcriptase enzyme.
- If desired, substitute 0.25 µL of SuperScript II or SuperScript III Reverse Transcriptase (provided by the user) for the AccuLift Reverse Transcriptase. However, do not use the SuperScript 5X Buffer or the DTT that is provided with the SuperScript enzymes.

To each reaction from Part B Step 3, add:

Component	Volume (µL)
Message*BOOSTER* Reverse Transcription PreMix	1.25
DTT	0.25
RiboGuard RNase Inhibitor	0.25
MMLV Reverse Transcriptase	0.25
Total reaction volume	**6**

5 Gently mix the reactions. If using AccuLift Reverse Transcriptase or SuperScript II Reverse Transcriptase, incubate the reactions at 37 °C for 60 min. If the thermocycler has a heated-lid function, heat the lid only if the temperature of the lid can be maintained at 37 °C.

If using SuperScript III Reverse Transcriptase, incubate the reactions at 50 °C for 30–60 min. If the thermocycler has a heated-lid function, heat the lid only if the temperature of the lid can be maintained at 50 °C.

Part C: Round-One, Second-Strand cDNA Synthesis

Required in Part C

Component Name	Tube Label	Cap Color
DNA Polymerase PreMix	DNA Polymerase PreMix	Blue
DNA Polymerase 1	DNA Polymerase	
cDNA Finishing Solution	cDNA Finishing Solution	

Incubation temperatures performed in Part C: 37°C, 65°C, and 80°C.

1 To each reaction from Part B, Step 5, add:

Component	Volume (µL)
DNA Polymerase PreMix	4.5
DNA Polymerase	0.5
Total reaction volume	**11.0**

2 Gently mix the reaction(s) and then incubate at 65 °C for 10 min in a water bath or thermocycler. Centrifuge briefly in a microcentrifuge.

IMPORTANT Be sure to incubate the reactions at 65 °C.

3 Terminate the reactions by heating at 80 °C for 3 min. Centrifuge briefly in a microcentrifuge, then chill on ice.

4 Add 1 µL of cDNA Finishing Solution to each reaction.

5 Gently mix the reactions and then incubate at 37 °C for 10 min.

6 Transfer the reaction(s) to 80 °C and incubate for 3 min.

7 Centrifuge briefly in a microcentrifuge, then chill on ice.

NOTE If desired, the reactions can now be frozen and stored overnight at –20 °C.

Part D: *In Vitro* Transcription

Required in Part D:

Component Name	Tube Label	Cap Color
In Vitro Transcription PreMix	IVT PreMix A	Green
T7 RNA Polymerase	T7 RNA Polymerase	
T7 Transcription Buffer	10X Transcription Reaction Buffer	
RNase-Free DNase I	RNase-Free DNase I	
Dithiothreitol	DTT	Clear

Incubation temperatures performed in Part D: 37 °C and 42 °C.

1 Warm the T7 RNA Polymerase to room temperature. Thaw the remaining *in vitro* transcription reagents at room temperature.

2 Thoroughly mix the thawed T7 Transcription Buffer.

IMPORTANT If a precipitate is visible in the thawed AccuLift T7 Transcription Buffer, heat the Buffer to 37 °C and mix well until it dissolves. Mix the Buffer thoroughly.

3 To each reaction from Part C, Step 6, add:

Component	Volume (µL)
T7 Transcription Buffer	2
In Vitro Transcription PreMix	14
DTT	2
T7 RNA Polymerase	2
Total reaction volume	**32**

4 Gently mix the reaction(s) and then incubate at 42 °C for 4 hours in a thermocycler or a water bath. If the thermocycler has a heated-lid function, heat the lid only if the temperature of the lid can be maintained at 42 °C.

IMPORTANT Do not exceed 4 hr incubation. The 4-hr incubation gives optimal RNA yield and quality (length). Longer incubation times will result in lower quality RNA.

5 Add 2 µL of RNase-Free DNase I to each reaction. Mix gently and then incubate each reaction at 37 °C for 15 min.

Part E: RNA Purification

Purification of the amplified RNA is necessary prior to the final cDNA synthesis step. We recommend purification of the amplified RNA using the AccuLift RNA Cleanup and Concentrator columns (PN 10012; provided by the user). These columns elute the purified RNA in a volume that is compatible with the final cDNA synthesis step (Part F). Other commercial RNA purification columns can be used (for example, RNeasy MinElute Cleanup Kit; Qiagen, Cat. No. 74204) but their use may require reducing the volume of the eluted RNA prior to the Round-Two, First-Strand cDNA Synthesis step.

If using the RNA Cleanup and Concentrator columns, follow the manufacturer's procedure for purifying Total RNA including small RNAs with the following suggestions:

- The volume of a reaction from Part D, Step 5 (above) is 34 µL. Therefore, in Step 1 of the RNA Cleanup and Concentrator column procedure, add 68 µL of RNA Binding Buffer to each sample and mix.
- In Step 7 of the AccuLift RNA Cleanup and Concentrator column procedure, elute the RNA using 8 µL of RNase-Free Water. Apply the water directly onto the center of the column membrane. Wait 5 min. Centrifuge at full speed for 1 min to collect the amplified RNA. The eluted RNA can be used immediately in Part F.

If using the RNeasy MinElute Cleanup Kit (Qiagen) columns:

- Add 0.5 µL of the Poly(I) (provided in the) to each sample.
- Follow the manufacturer's directions for sample application and washing.
- Elute the RNA from the column using 14 µL of RNase-Free Water. Then, reduce the volume of the eluted RNA to 3–8 µL using a Speed-Vac® Concentrator. Do not let the sample dry.

  NOTE If desired, the amplified RNA can now be quick-frozen (for example, in a dry ice/ethanol bath) and stored overnight at –70°C to –80°C.

Part F: Round-Two, First-Strand cDNA Synthesis

The purified RNA should be in a volume of 3-8 µL for optimal results. If the purified RNA is in a volume >8 µL, reduce the volume of the RNA using a Speed-Vac Concentrator. Do not let the sample dry.

AccuLift Reverse Transcriptase is provided in the kit. However, other MMLV RT-type enzymes such as SuperScript II or SuperScript III (provided by the user) can be used. Modifications to the protocol for use of either of the SuperScript enzymes are noted in the procedure.

Required Materials in Part F:

Component Name	Tube Label	Cap Color
Reverse Transcription PreMix	RT PreMix	Red
MMLV Reverse Transcriptase	MMLV-RT	
RiboGuard RNase Inhibitor	RiboGuard RNase Inhibitor	
Random Primers	Random Primers	Yellow
RNase H	RNase H	
Dithiothreitol	DTT	Clear

Incubation temperatures performed in Part F: 37 °C, 65 °C, and 95 °C.

1. To each sample, add 2 µL of the Random Primers.

2 Incubate at 65 °C for 5 min in a water bath or thermocycler to anneal the Random Primers to the RNA.

3 Chill on ice for 1 min. Centrifuge briefly in a microcentrifuge.

4 To each reaction, add:

Component	Volume (µL)
Reverse Transcription PreMix	1.25
DTT	0.25
RiboGuard RNase Inhibitor	0.25
MMLV Reverse Transcriptase	0.25
Primer	2
Purified RNA	3–8
Total Reaction Volume	**7–12**

IMPORTANT If using SuperScript II or SuperScript III Reverse Transcriptase, do not use the SuperScript 5X Buffer or the DTT that is provided with the SuperScript enzymes.

5 Gently mix the reaction(s) and then incubate each at room temperature for 10 min.

6 If using AccuLift Reverse Transcriptase or SuperScript II Reverse Transcriptase, incubate the reaction(s) at 37 °C for 1 hr in a water bath or thermocycler. If the thermocycler has a heated-lid function, heat the lid only if the temperature of the lid can be maintained at 37 °C.

If using SuperScript III Reverse Transcriptase, incubate the reaction(s) at 50 °C for 1 hr in a water bath or thermocycler. If the thermocycler has a heated-lid function, heat the lid only if the temperature of the lid can be maintained at 50 °C.

7 To each sample, add 0.5 µL of RNase H.

8 Gently mix each reaction and then incubate each at 37 °C for 20 min in a water bath or thermocycler. If the thermocycler has a heated-lid function, heat the lid only if the temperature of the lid can be maintained at 37 °C.

9 Transfer the reactions to 95 °C.

10 Incubate each at 95 °C for 2 min in a water bath or thermocycler.

11 Chill on ice for 1 min.

12 Centrifuge briefly in a microcentrifuge.

The cDNA produced, now in a volume of 7.5 µL-12.5 µL, does not need to be purified prior to qPCR. The cDNA can be diluted and used immediately for qPCR (see Appendix A; below, for details) or stored at –20 °C.

Appendix A: Guidelines for qPCR Using cDNA Produced by a cDNA Synthesis from Cell Lysates Kit Reaction

Primer selection

Since the first-strand cDNA synthesis is done using an oligo(dT) primer, we recommend selecting PCR primers that prime within 500 bases of the 3′ end of the mRNA. Primers for sequences >500 bases from the 3′end of the mRNA(s) may give reduced sensitivity.

qPCR using fluorescent probes or fluorescent dye detection

Either fluorescent probes or fluorescent dyes can be used successfully.

Dilution of the cDNA for qPCR

The cDNA dilution factor is dependent on the number of cells used in the reaction and the abundance of the transcript of interest. Typically, 1 µL of diluted cDNA is used in a 25 µL qPCR.

Use the following table as a guide to diluting the cDNA for qPCR. Dilutions of the cDNA should be made into TE buffer [10 mM Tris-HCl (pH 7.5), 1 mM EDTA; provided by the user].

Number of Cells in the Reaction	Dilution
1–10	1:2 to 1:100
10–50	1:10 to 1:100
50	1:50 to 1:1,000
>50	1:100 to 1:1,000

Appendix B: The Control Reaction: RNA Amplification and cDNA Synthesis

The kit provides 500 ng of NRK total RNA at a concentration of 50 ng/µL and forward and reverse PCR primers for the low-abundance PBGD mRNA.

Required for the Control Reaction:

Component Name	Tube Label	Cap Color
NRK Total RNA Control (50 ng/µL)	NRK Total RNA	Clear
Nuclease-Free Water	Nuclease-Free Water, Sterile	
Forward Control PCR Primer	Forward Control PCR Primer	Yellow
Reverse Control PCR Primer	Reverse Control PCR Primer	

T7-Oligo(dT) Primer	T7-Oligo(dT) Primer	Red

1. Thaw the NRK Total RNA Control on ice.
2. On ice, dilute the thawed NRK Total RNA Control 1:1,000 with Nuclease-Free Water by adding 1 µL of the NRK Total RNA Control to 999 µL of Nuclease-Free Water. The concentration of the diluted NRK Total RNA Control is now 50 pg/µL.
3. Anneal the T7-Oligo(dT) Primer to the diluted NRK Total RNA Control.

   The standard control reaction uses 50 pg of the NRK Total RNA Control.

Component	Volume (µL)
Nuclease-Free Water	2
Diluted NRK Total RNA Control (50 pg)	1
T7-Oligo(dT) Primer	1
Total reaction volume	**4**

4. Incubate the reaction at 65 °C for 5 min in a water bath or thermocycler. While the reaction incubates, quick-freeze the remaining undiluted NRK Total RNA Control, for example in a dry ice/ethanol bath, and return it to storage at –70 °C to –80 °C. Discard the diluted NRK Total RNA Control.
5. Cool the annealing reaction on ice for at least 1 min. Centrifuge the tube for 5–10 sec to bring the sample to the bottom of the tube.
6. Continue the Control Reaction as described beginning in Part B, Step 4 through Part F. Then, perform the Control qPCR as described in Appendix C.

Appendix C: The AccuLift Ultra-sensitive RNA Amplification Kit Control Reaction: The Control qPCR

Included in the AccuLift Ultra-sensitive RNA Amplification Kit are the Forward and Reverse Control PCR primers for PCR amplification of the low-abundance PBGD mRNA present in the NRK Total RNA Control. Use of these primers with cDNA produced from RNA other than the provided NRK Total RNA Control may give unexpected results or results that are difficult to evaluate.

Required for the Control qPCR:

Component Name	Tube Label	Cap Color
Forward Control PCR Primer	Forward Control PCR Primer	Yellow
Reverse Control PCR Primer	Reverse Control PCR Primer	Yellow
Nuclease-Free Water	Nuclease-Free Water, Sterile	Clear

1. Using the cDNA produced from the NRK Total RNA Control in Part F, Step 9, set up qPCRs. Mix the following reagents in PCR tubes:

Component	Volume (µL)
Nuclease-Free Water	9.5
Forward Control PCR Primer	1
Reverse Control PCR Primer	1
2X qPCR MasterMix (provided by the user)	12.5
cDNA	1
Total reaction volume	**25**

2. Place the tubes in a real-time PCR thermocycler. Use the cycling program recommended by the thermocycler and qPCR MasterMix manufacturer. We recommend running a 40-cycle program using a two-step protocol annealing and extending at 60 °C for 30 sec. Melting curve analysis should be performed.

   If using fluorescent dye detection, melting curve analysis should be performed. Using the Control Primers included in the kit, melting curve analysis shows a sharp peak at Tm = 88 °C.

Appendix D: 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.

For support visit fluidigm.com/acculift-lcm.

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