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Arcturus® RiboAmp® PLUS RNA Amplification Kit

User Guide

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About This Guide

Purpose of this guide

This user guide is for use with Arcturus® RiboAmp® PLUS RNA Amplification Kit. You can view and download this user guide from: **www.appliedbiosystems.com**

Prerequisites

This guide is intended for those who use the RiboAmp® PLUS RNA Kit. Life Technologies is not liable for damage or injury that results from use of this manual by unauthorized or untrained parties. Instructions in this guide use conventions and terminology that assume a working knowledge of the Microsoft® Windows® operating system, the Internet, and Internet-based browsers.

Safety information

For general safety information, see this section. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the Safety appendix at the end of the manual for the complete alert on the chemical.

Four safety alert words appear in this user documentation at points in the document where you need to be aware of relevant hazards. Each alert word implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for accurate chemistry kit use, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Except for IMPORTANTs, each safety alert word in an Applied Biosystems document appears with an open triangle figure that contains a hazard symbol.

1 Product Introduction

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Background information

The Arcturus® RiboAmp® PLUS RNA Amplification Kit enables you to produce microgram quantities of antisense RNA (aRNA) from nanogram quantities of total cellular RNA. Small sample collection methods, such as needle aspiration and Laser Capture Microdissection (LCM), limit starting quantities of RNA available for amplification. Microarray hybridization experiments require microgram quantities of probe per array to enable detection, and most studies entail multiple experiments.

High yields

The RiboAmp® PLUS RNA Kit achieves high yields of aRNA with a patented linear amplification process using double-stranded cDNA as template in a T7 RNA polymerase catalyzed amplification. This amplification process yields highly reproducible results through an optimized system of reagents, purification devices, and protocols. The resulting aRNA is suitable for use in RT-PCR, quantitative Real-Time PCR (qRT-PCR), and microarray hybridization experiments.

The Arcturus® RiboAmp® PLUS RNA Kit can generate different forms of aRNA:

- Unlabeled aRNA, ready for reverse transcription and quantitative Real-Time PCR (qRT-PCR).
- Amino-allyl incorporated aRNA, ready to be labeled for microarray analysis.
- Labeled aRNA (when coupled with the Arcturus® Turbo Labeling™ Kit), which is then ready for microarray experiments.

Kit contents

The kit contains:

- A complete set of reaction reagents.
- Nucleic acid purification columns.
- A control RNA sample.
- This user guide.

Reagents and materials are supplied for 12 one-round amplifications or 6 two-round amplifications. The kit provides premixed enzymes and buffers to save time and increase ease of handling. The protocol is streamlined to enable fast processing while generating reproducible results.

aRNA product size

The kit generates aRNA product that is shorter than the starting mRNA template. The bulk of the aRNA product is 250–1800 bases in length after one round of amplification and slightly shorter (under 200 to over 600 bases) after a second round. Messenger RNA makes up an estimated 1-5% of total cellular RNA. One round of amplification typically yields up to 1000-fold amplification of the mRNA, while two rounds may yield up to one-million fold amplification of the mRNA. Amplified aRNA produced using the RiboAmp® PLUS Kit is ready for subsequent labeling and probing of cDNA microarrays.

Note: RNA amplification begins at the 3′ end of the substrate molecule; therefore, amplified RNA product should not be used to prepare full-length cDNA libraries.

IMPORTANT! Microarray experiments that compare labeled RNA amplified through linear amplification to labeled unamplified RNA should be designed to compare differential gene expression using RNA samples that are processed using identical methods.

Performance specifications

One round of amplification using a recommended input quantity of Control RNA supplied with the RiboAmp® PLUS RNA Kit typically yields about 30-50 µg of aRNA.

Master Mix quantity

The RiboAmp® PLUS RNA Kit is designed with the assumption that you will use the mix when you need three or more samples, and that you will not use it for two or fewer samples. The kits have been calculated to have a 10% overage for three samples. If you exceed 10% overage for master mixes, this may result in insufficient material to complete all six reactions. A suggested master mix size for six samples is included when appropriate.

RNA input requirements and comparisons

The two tables in this section provide the total RNA input requirements for RiboAmp® PLUS RNA Amplification (Table 1), and the differences between the RiboAmp® PLUS RNA Amplification Kit and the RiboAmp® HS PLUS RNA Amplification Kit (Table 2).

Table 1 Total RNA input requirements for RiboAmp® PlUS DNA Amplification

RiboAmp® PLUS Input Amounts*	Minimum†	Recommended‡
RiboAmp® PLUS 1-round	500 ng	2 µg–10 µg
RiboAmp® PLUS 2-round	5 ng	10 ng–40 ng
RiboAmp® HS PLUS 2-round	100 pg**	500 pg–5 ng

* Input required to obtain sufficient aRNA yields for microarray hybridizations. If your application is anything other than microarray, please contact us for input recommendations.

† Minimum input will yield >15 µg of aRNA.

‡ Recommended input will yield > 30 µg of aRNA.

** The RiboAmp® HS PLUS kit has been validated down to 100 pg. However new users should start with the control RNA provided with the kit until they become comfortable with the use of the kit.

Note: Do not overload amplification reactions. Overloading high sensitivity kits may deplete key components during the reaction and may lead to no amplification.

Table 2 Differences between the RiboAmp® HS PLUS RNA Kit and RiboAmp® Plus RNA Kit

RiboAmp® HS PLUS RNA Kit	RiboAmp® PLUS RNA Kit
Higher sensitivity	Faster processing
2 rounds of amplification	1 or 2 rounds of amplification
Recommend 500 pg–5 ng total RNA input	Recommend 5–40 ng total RNA input for 2 rounds of amplification
Requires 2.5 days	Requires ~13 hours total for 2 rounds

Storage and stability

Life Technologies makes recommendations for storage temperatures throughout this document. Realizing that not every laboratory has a freezers set at these temperatures, we have defined the acceptable temperature ranges for our recommendations.

Acceptable storage ranges

Acceptable ranges for storage are:

- -70°C = -65°C to -80°C
- -20°C = -15°C to -30°C
- 4°C = 2°C to 8°C
- Room Temperature = 10°C to 30°C

Recommended storage temperatures

The RiboAmp® PLUS kits have both room temperature and frozen components. The room temperature components should be stored at normal room temperature. The frozen components are shipped on dry ice and should be stored at -70°C until initial use. After initial use, we recommend -20°C to prevent unnecessary freeze-thaws of the enzymes. The control RNA and any RNA generated from RiboAmp® PLUS RNA amplification kit should always be stored at -70°C.

Store the frozen reagent box at -70°C in a non frost-free freezer upon receipt. After the initial use of the frozen reagents, storage at -20°C is recommended. The reason to store the kit at -20°C instead of at -70°C is to prevent unnecessary freeze-thaw of the enzymes. The Control RNA vial should be stored at -70°C or below immediately upon arrival to ensure maximum stability.

Store the room temperature box at room temperature.

Expiration

All reagents included with the system should be used within one year of receipt. For optimal results, use the reagents as soon as possible after receipt.

2 Kit Components

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Kit configurations

The table below lists the Arcturus® RiboAmp® PLUS RNA Amplification Kit configurations. The catalog numbers are provided. These kits can be ordered online.

Table 3 RiboAmp® PLUS RNA Amplification Kit Configurations

Description	Catalog Number	Number of Samples	cDNA	Amplification	IVT	AA-IVT Purification	Labeling
RiboAmp® PLUS	KIT0521	6	1x RA7016	1x RA7011	1x RA7009		
RiboAmp® PLUS with Biotin Labeling	KIT0511-B	12	2x RA7016	2x RA7011	2x RA7009		1x KIT0608
RiboAmp® PLUS with CY®3 Labeling	KIT0511-C	12	2x RA7016	2x RA7011	2x RA7009		1x KIT0609
RiboAmp® PLUS with CY®5 Labeling	KIT0511-D	12	2x RA7016	2x RA7011	2x RA7009		1x KIT0610
RiboAmp® PLUS Amino Allyl	KIT0521aa	6	1x RA7016	1x RA7011	1x RA7010	1x RA7012	
RiboAmp® Plus (bulk)	KIT0501	24	4x RA7016	4x RA7011	4x RA7009		
RiboAmp® PLUS Amino Allyl (bulk)	KIT0501aa	24	4x RA7016	4x RA7011	4x RA7010	4x RA7012	
RiboAmp® PLUS 1.5 Rounds	KIT0526	6	1x RA7016	1x RA7011	1x RA7008		
RiboAmp® PLUS 0.5 Rounds (bulk)	KIT0527	12	4x RA7016	4x RA7011			

Contents of modules

The tables in this section list the contents of the various modules.

cDNA module

Table 4 RiboAmp® PLUS–RA7016

Component	Vial Color	Vial Label
1st Strand Master Mix	Red	1
1st Strand Enzyme Mix	Red	2
Enhancer	Yellow	E
Nuclease Mix	Orange	
2nd Strand Master Mix	Green	1
2nd Strand Enzyme Mix	Green	2
Primer A	Grey	A
Primer B	Grey	B
Control RNA	Purple	C

In Vitro Transcription (IVT) modules

Table 5 In Vitro Transcription (IVT) 1-round–RA7008

Component	Vial Color	Vial Label
IVT Buffer	Blue	1
IVT Master Mix	Blue	2
IVT Enzyme Mix	Blue	3
DNase Mix	Blue	4

Table 6 In Vitro Transcription (IVT) 2-round–RA7009

Component	Vial Color	Vial Label
IVT Buffer	Blue	1
IVT Master Mix	Blue	2
IVT Enzyme Mix	Blue	3
DNase Mix	Blue	4

Table 7 Amino-allyl IVT–RA7010

Component	Vial Color	Vial Label
IVT Buffer	Blue	1
IVT Master Mix	Blue	2
IVT Enzyme Mix	Blue	3
DNase Mix	Blue	4

Component	Vial Color	Vial Label
Amino-allyl IVT Master Mix	Teal	AA
Labeling Buffer	Teal	LB
DMSO	Teal	DMSO

DNA / RNA purification modules

Table 8 cDNA/aRNA Purification–RA7011

Component	Vial Color	Vial Label
DNA Binding Buffer	Red	DB
DNA Wash Buffer	Red	DW
DNA Elution Buffer	Red	DE
RNA Binding Buffer	Blue	RB
RNA Wash Buffer	Blue	RW
RNA Elution Buffer	Blue	RE
0.5 mL Microcentrifuge Tubes		
Purification columns with collection tubes		

Table 9 Amino-allyl aRNA Labeling Purification–RA7012

Component	Vial Color	Vial Label
RNA Binding Buffer	Blue	RB
RNA Wash Buffer	Blue	RW
RNA Elution Buffer	Blue	RE
0.5 mL Microcentrifuge Tubes		
Purification columns with collection tubes		

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Recommendations for RNase-free technique

RNase contamination will cause experimental failure. Minimize RNase contamination by adhering to the following recommendations throughout your experiment:

- Wear disposable gloves and change them frequently.
- After putting on gloves, avoid touching surfaces that may introduce RNases onto the glove surface.
- Do not use reagents not supplied in the RiboAmp® PLUS Kit or recommended for use within this guide. Substitutions of reagents or kit components may adversely affect yields or introduce RNases.
- Use only new, sterile RNase-free pipette tips and microcentrifuge tubes.
- Clean work surfaces with commercially available RNase decontamination solutions prior to performing reactions.

Amplified aRNA contamination

Stray amplified aRNA in the work area can contaminate precious samples if the work area is routinely used for performing amplifications. To ensure a work area free of amplified aRNA:

- Irradiate the work area/hood with UV overnight every three to four days.
- Clean surfaces with commercially available decontamination solutions.

RNA Preparation

RNA Quality

The success of amplification using the RiboAmp® PLUS RNA Amplification Kit depends on the quality of the source RNA. Integrity is affected by exposure to internal and external sources of RNases. Avoiding RNA degradation due to intracellular RNases is often the most critical step in isolating good quality RNA. Isolation from cell cultures should be performed immediately after harvesting the cell to avoid RNase activity. Quiescent cells, such as those in tissue samples, require immediate freezing in embedding media to inactivate RNases. Subsequent processing of those samples requires methods that preserve RNA integrity.

We recommend the use of the PicoPure® RNA Isolation Kit for isolating RNA from tissue or cell samples. Cells captured by LCM will yield high quality RNA when appropriate protocols are applied, such as those used in the Arcturus® HistoGene® LCM Frozen Section Staining Kit, the HistoGene® LCM Immunofluorescence Staining Kit and PicoPure® RNA Isolation Kit.

We strongly recommend performing quality assessment of the tissue prior to amplification. Tissue that has degraded RNA prior to freezing will not yield good results. If the quality of the source tissue is unknown, then performing a quality assessment of the tissue block prior to spending the time and expense of Laser Capture and amplification is imperative. A protocol for quality assessment is included in Appendix B, "aRNA Analysis" in this document.

RNA Input

Using isolated total cellular RNA, rather than mRNA, for amplification is recommended to reduce the loss of valuable transcripts during mRNA isolation from extremely small samples. Isolating RNA with the PicoPure® RNA Isolation Kit is also highly recommended. Furthermore, input RNA should be DNase-treated to eliminate genomic DNA contamination. The RNA must be provided in RNase-free water, without the presence of organic solvents, salts, or contaminating cellular material.

IMPORTANT! The RiboAmp® PLUS RNA Amplification kits are not designed for use with formalin-fixed material. For Formalin-Fixed materials please use the Arcturus® Paradise® PLUS Reagents System.

RNA Storage

RNA intended for use with the RiboAmp® PLUS Kits should be used immediately after isolation or stored at -70°C until use. The control RNA provided with each RiboAmp® PLUS Kit should be stored at -70°C immediately upon arrival. Avoid multiple freeze-thaw cycles.

Amplified aRNA produced using the RiboAmp® PLUS Kit should be used for labeling reactions as soon as possible. Alternatively, the aRNA may be stored at -70°C.

Additional Lab Equipment and Materials Required

This is the required equipment:

- Thermal cycler with heated lid
- Microcentrifuge for 1.5 mL and 0.5 mL tubes (Eppendorf® 5414D or similar)
- 0.5–10 µL pipettor
- 20 µL pipettor
- 200 µL pipettor
- 1000 µL pipettor
- Ice bath or cold block (4°C)
- Vortex mixer (optional)

These are the required materials:

- 0.5 mL or 0.2 mL RNase-free microcentrifuge tubes
- 2 mL lidless tube for centrifuge (PGC Scientific, Cat # 16-8101-06)
- Nuclease-free pipette tips

4 RiboAmp® PLUS Amplification

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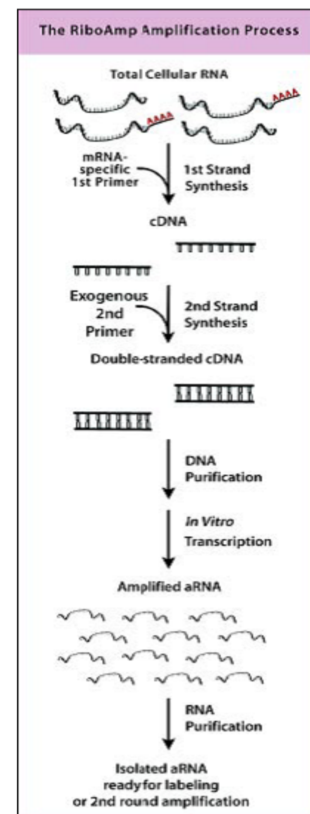
Overview of the amplification process

The Arcturus® RiboAmp® PLUS RNA Amplification Kit is optimized to amplify nanogram amounts of starting RNA. The RiboAmp® PLUS Kit utilizes up to two rounds of a five-step process for linear amplification of the mRNA fraction of total cellular RNA. These five steps are listed below and shown in Figure 1.

1. 1st strand synthesis reaction that yields cDNA incorporating a T7 promoter sequence.
2. 2nd strand synthesis reaction utilizing exogenous primers that yields double-stranded cDNA.
3. cDNA purification using specially designed MiraCol™ Purification Columns.
4. In vitro transcription (IVT) utilizing T7 RNA polymerase yields antisense RNA (aRNA).
5. aRNA isolation with the MiraCol Purification Columns.

The RiboAmp® PLUS Kit allows for up to two rounds of amplification for each sample. In vitro transcription can be performed overnight with the proper thermal cycler programming. The entire process can be completed by the morning of the third day, and the product is then ready for labeling and microarray hybridization or qRT-PCR.

Figure 1 RiboAmp® PLUS RNA Amplification Process



Time requirements

The table below presents typical time requirements for completion of the protocol. Times reflect total handling and reaction times of each step. Note that although there are safe stopping points for pausing the amplification process, the times presented reflect a continuous, uninterrupted process.

Table 10 Time Required for RiboAmp® PLUS

Steps	1st Round (hours)	2nd Round (hours)
1st Strand Synthesis	1	1
2nd Strand Synthesis	0.5	0.5
cDNA Purification	0.5	0.5
Total (before IVT)	2	2
In vitro transcription	3	4.5
aRNA Purification	0.5	0.5
Total	5.5	7

Thermal cycler programming

Thermal cyclers provide a convenient and reproducible method of incubating reactions according to specified temperatures and times in the RiboAmp® PLUS Kits protocol. A thermal cycler program for use with the RiboAmp® PLUS Kit appears below. The program is not intended for automatic progression from one time and temperature set to another. The program lists a 4–8°C hold after each incubation or incubation cycle when it is necessary to remove the reactions from the thermal cycler to add reagents. After the addition of reagents, place the sample back into the thermal cycler and resume the program.

IMPORTANT! Using a thermal cycler with a heated lid is important. The heated lid ensures proper temperature distribution within the reaction tube and prevents evaporative condensation that alters the reaction mixture concentrations.

The 4–8°C steps in the thermal cycler program allow for buffer and reagent addition and mixing steps at certain points during the amplification process and are not intended for indefinite hold unless noted.

The thermal cycler should be calibrated to the manufacturer's specifications.

Table 11 RiboAmp® PLUS Thermal Cycler Program Round One

	ºC	Time
1st Strand Synthesis	65ºC	5 minutes
	4ºC	hold
	42ºC	45 minutes
	4ºC	hold
	37ºC	20 minutes
	95ºC	5 minutes
	4ºC	hold

2nd Strand Synthesis	95ºC	2 minutes
	4ºC	hold
	25ºC	5 minutes
	37ºC	10 minutes
	70ºC	5 minutes
	4ºC	hold
IVT	42ºC	3 hours (optional 4 hours)
	4ºC	hold (optional overnight hold)
	37ºC	15 minutes
	4ºC	hold

Table 12 RiboAmp® PLUS Thermal Cycler Program Round Two

	ºC	Time
1st Strand Synthesis	65ºC	5 minutes
	4ºC	hold
	25ºC	10 minutes
	37ºC	45 minutes
	4ºC	hold
2nd Strand Synthesis	95ºC	2 minutes
	4ºC	hold
	37ºC	15 minutes
	70ºC	5 minutes
	4ºC	hold
IVT	42ºC	4-6 hours
	4ºC	hold (optional overnight hold)
	37ºC	15 minutes
	4ºC	hold

IMPORTANT! Do not allow incubation times and temperatures to deviate from the protocol.

Necessary preliminaries

Before you begin Round One of the thermal cycling, review the recommendations and instructions provided here.

Protocol notes

- When adding reagent to samples or master mixes, pipette mixtures up and down several times to ensure complete transfer of reagent from the pipette tip.
- Prior to the first use of an enzyme, gently mix (do not vortex) and briefly microcentrifuge the vial to ensure that all enzyme is mixed and collected at the bottom of the vial. Enzyme may collect on the vial wall or cap during shipment.
- Keep thawed reagents and reaction tubes in cold blocks at 4–8°C while adding reagents to samples.
- Prior to each incubation, mix samples thoroughly by flicking the reaction tube (unless noted in protocol) to ensure optimal performance. Spin down before proceeding. *Do not vortex*.
- Use a microcentrifuge to spin down all components and samples following each mixing step.
- Clean all amplification process equipment with an RNase eliminator such as RNase AWAY® (Molecular Bio Products) to minimize the risk of RNase contamination.
- During enzyme and buffer dispensing, keep the reaction tube with sample on ice or chilled in a 4–8°C cold block. Do not freeze samples unless it is indicated in the protocol.

Preparing samples and reagents

Note: Although excess enzyme and reagents are provided in all vials, there is insufficient volume to prepare extra reactions.

1. Thaw the frozen kit components as needed, and mix by flicking the tube or by inverting the tubes several times, spin down, and place on ice. When enzyme mixtures have been removed from -20°C storage for use, always keep them in a cold block or in an ice bucket at the lab bench.
2. Allow In Vitro Transcription (IVT) Buffer (Blue-labeled Vial 1), Master Mix (Blue labeled Vial 2) and Enhancer (Yellow-labeled Vial) to assume room temperature, and mix by inverting or flicking the tube. Spin down if necessary. Dissolve all visible solids prior to use.
3. Isolate RNA from LCM samples using the PicoPure® RNA Isolation Kit (KIT0204) for best results.

   Note: Avoid using organic solvents in RNA isolation procedures. Trace amounts of organic solvents that carry over into amplification reactions will impair the RiboAmp® PLUS Kit amplification process.
4. First, DNase-treat the RNA prior to putting it into the RiboAmp® PLUS Kits to eliminate possible genomic DNA interference in the amplification process. You must then remove the DNase enzyme and buffers prior to putting the RNA into the RiboAmp® PLUS Kits protocol.

   Note: You can incorporate DNase treatment directly into the protocol of the PicoPure® RNA Isolation Kit or other purification column-based approaches.

   Note: Using nucleic acid carrier is not necessary.

Using spin columns for nucleic acid elution

Spin columns and 0.5-mL microcentrifuge tubes are provided for nucleic acid elution. Improper orientation of tubes during centrifugation may result in cap breakage or sample loss.

To use the column-tube assembly correctly:

1. Insert a spin column into the 0.5-mL tube, aligning the two cap hinges as illustrated.
2. Load Elution Buffer onto the column and incubate as directed.
3. Place the column-tube assembly into a 2-mL lidless support tube (PGC Scientific, Catalog #16-8101-06) in the centrifuge rotor; alternately, retain and reuse the 2 mL lidless collection tubes provided.

   Note: Some varieties of 2-mL tubes will not provide enough support. Contact Life Technologies Technical Support for other alternatives. Call 1-800-831-6844.

4. Skip one rotor position between assemblies, and position assemblies with the 0.5-mL tube cap trailing the tube during centrifugation as shown in Appendix D, “Centrifuge Information”.
5. Check for a mark on the centrifuge indicating rotation direction.
6. Centrifuge as directed in the protocol.

Using the control templates

A control RNA sample (Purple) is provided along with each kit to be used as a control template to verify amplification efficacy. Use 10 µL of this RNA for control amplifications.

For RiboAmp® PLUS, 10 µL of control RNA contains 2 µg (200 ng/µL) of total RNA. This is enough RNA to be used as a control for one round amplifications. For use as a 2-round control, you may adjust input to lower amounts (1-4 ng/ µL). When diluting control RNA, use 10 ng/µL Poly I to perform dilutions.

Avoiding contamination

Due to the sensitivity of the RiboAmp® PLUS Kits, it is very important to prevent RNA, DNA, and nuclease contamination. Clean work surfaces before and after each use. Perform all dispensing in a work hood that has been irradiated with UV to remove contaminants from previous amplification experiments.

5 RiboAmp® PLUS Protocol

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RiboAmp® PLUS Round One

Round One: 1st strand cDNA synthesis

IMPORTANT! Read all of the notes on the previous chapter prior to beginning.

Note: Do not leave reagents at room temperature. Place components back onto the cold block or refreeze immediately after dispensing the reagent.

1. Prepare RNA sample in a total volume of 10–11 µL in a 0.5- or 0.2-mL RNase-free microcentrifuge tube and place on 4–8°C block. For recommended sample input quantities, see "RNA input requirements and comparisons" on page 11.
2. Add 1.0 µL of Primer A (Grey-A), mix well, and spin down.
3. Incubate at 65°C for 5 minutes then chill the samples to 4°C for at least 1 minute. Hold the sample at 4°C until ready to proceed.
4. Spin the contents down before proceeding to the next step.
5. Thaw 1st Strand Synthesis components (Red-labeled Vials) and place on ice.

   Note: 1st Strand Enzyme Mix (Red-2) does not require thawing and can be placed directly on ice.
6. Add reagents directly to the sample.

   Note: If you are performing several amplifications, you will find it more convenient to prepare Complete 1st Strand Synthesis Mix according to Table 13.

Table 13 Round One first-strand reaction mix.

Complete 1st Strand Synthesis Mix			6 reaction Master Mix with 10% overage
Component	Amount	Vial #	
1st Strand Master Mix	7 µL	Red-1	46.2 µL
1st Strand Enzyme Mix	2 µL	Red-2	13.2 µL
Total per sample	9 µL	—	59.4 µL

7. Incubate at 42°C for 45 minutes, then chill the sample to 4–8°C for at least 1 minute. Do not hold samples at this step for a prolonged period of time. Keep samples at 4–8°C until next incubation.

8. (Optional) You can remove a 2.0-µL sample at this point in the protocol to assess the integrity of the starting mRNA by qRT-PCR. (This may reduce your final yield.)
9. Thoroughly mix and spin down Nuclease Mix (orange). Place on ice.
10. Add 2.0 µL of Nuclease Mix to the sample, and mix thoroughly by flicking the tube, and then spin down.
11. Incubate the sample at 37°C for 30 minutes followed by 95°C for 5 minutes.
12. Chill the sample to 4–8°C for at least 1 minute.

Note: It is safe to stop at this point in the protocol. You can store the sample overnight at -20°C.

Round One: 2nd strand cDNA synthesis

1. Place sample on 4–8°C block, and allow to thaw if frozen (at 4–8°C).
2. Thaw Primer B (Gray-B), mix thoroughly, and spin down.
3. Add 1.0 µL of Primer B. Mix thoroughly by flicking the tube, and spin down.
4. Incubate sample at 95 °C for 2 minutes, then chill, and maintain the sample at 4–8°C for at least 2 minutes.
5. Thaw 2nd Strand Master Mix at 4–8°C (cold block) (Green-1). Mix thoroughly, and spin 2nd Strand Master Mix.
6. Mix enzyme thoroughly by inverting several times, spin briefly, and then place at 4–8°C.

   Note: 2nd Strand Enzyme Mix (Green-2) does not require thawing.
7. Add 2nd Strand Synthesis components separately in the order listed in the following table.

   If you are performing several amplifications, you may want to prepare a Complete 2nd Strand Synthesis Mix based on Table 14, and add 30 µL Complete 2nd Strand Synthesis Mix to each sample. Mix thoroughly by flicking the tube, and spin down. Store at 4°C until needed.

Table 14 Round One second-strand reaction mix

Complete 2nd Strand Synthesis Mix			6 reaction Master Mix with 10% overage (µL)
Component	Amount (µL)	Vial #	
2nd Strand Master Mix	29 µL	Green-1	191.4 µL
2nd Strand Enzyme Mix	1 µL	Green-2	6.6 µL
Total per sample	30 µL	—	198 µL

IMPORTANT! Place components back onto the cold block or refreeze immediately after dispensing the reagent. Do not leave reagents at room temperature for any extended period of time.

8. Incubate the sample as follows:
   - 25°C: 5 minutes
   - 37°C: 10 minutes

- 70°C: 5 minutes
- 4–8°C: Hold until ready to proceed (up to a maximum of 30 minutes)

Round One: cDNA purification

Note: DNA Binding Buffer (Red-DB) must be at room temperature and mixed thoroughly by shaking before use. A precipitate may form during long-term storage. Dissolve precipitate prior to use by mixing. If necessary, warm the DB vial to redissolve

1. Add 250 µL of DNA Binding Buffer (Red-DB) to a DNA / RNA Purification Column seated in the collection tube provided. Hold for 5 minutes at room temperature.
2. Centrifuge at 16,000 x g for 1 minute.
3. Add 200 µL of DNA Binding Buffer to the 2nd Strand Synthesis sample tube, mix well, and pipet the entire volume into the purification column.
4. To bind cDNA to column, centrifuge at 100 x g for 2 minutes (or lowest speed setting available), immediately followed by a centrifugation at 10,000 x g for 1 minute to remove flow-through.
5. Add 250 µL of DNA Wash Buffer (Red-DW) to the column and centrifuge at 16,000 x g for 2 minutes. Check the purification column for any residual wash buffer. If any wash buffer remains, re-centrifuge at 16,000 x g for 1 minute.
6. Discard the flow-through and collection tube.

   IMPORTANT! Avoid splashing flow-through in the collection tube onto the column. If flow-through waste liquid wets the outside of the purification column, re-centrifuge the column at 16,000 x g to remove liquid.
7. Place the column into the provided 0.5-mL microcentrifuge tube and carefully add 11 µL of DNA Elution Buffer (Red-DE) onto the center of the purification column membrane. (Gently touch the tip of the pipette to the surface of the membrane while dispensing the elution buffer to ensure maximum absorption of DE into the membrane).
8. Gently tap the purification column to distribute the buffer, if necessary. Incubate for 1 minute at room temperature.
9. Place the assembly into the centrifuge as shown, and centrifuge at 1000 x g for 1 minute, and then centrifuge at 16,000 x g for 1 minute.
10. Discard the column and retain the elution containing the cDNA in the microcentrifuge tube for further processing.

IMPORTANT! To avoid potential breakage of the microcentrifuge tube cap during centrifugation, insert the purification tube/0.5-mL tube assembly into a lidless 1.7/2.0 mL tube. Insert this assembly into adjacent rotor holes as illustrated. Rest the tube cap against the tube immediately clockwise to it. Place an empty, lidless 1.7/2.0-mL tube into the rotor hole adjacent in the clockwise direction to the last assembly.

Note: It is safe to stop at this point in the protocol. You can store the sample overnight at -20°C.

IMPORTANT! 0.5 Round Users Using Alternate IVT Labeling Procedures: *Stop here.*

0.5 Round users (KIT0527) using alternative IVT labeling (such as Affymetrix labeling kit 900449), *stop here*, and proceed to Appendix C, "Generating Labeled aRNA Using Alternative IVT Kits".

Note: Life Technologies recommends the Turbo Labeling™ microarray kit (Biotin - KIT0608, CY®3 - KIT0609, CY®5 - KIT0610) for higher yields and longer aRNA fragments. The Turbo Labeling™ kit allows for a more efficient labeling process leading to a better representation of the mRNA transcript and higher present calls.

Round One: In Vitro Transcription

1. Thaw IVT Buffer (Blue-1), Master Mix (Blue-2) and Enhancer (Yellow-E) to room temperature and thoroughly mix to dissolve all solids. (IVT Enzyme Mix (Blue-3) does not require thawing and can be put in directly at 4–8°C.)
2. Mix enzyme thoroughly by inverting several times. Spin briefly.

   Note: IVT reaction components must be thawed, thoroughly mixed with all solids dissolved, and brought to room temperature just before use.
3. Add IVT components in the order listed in the following table.

   Note: If you are performing several amplifications, you may want to prepare a Complete IVT Reaction Mix according to this table, and add 12 µL Complete IVT Reaction Mix to each sample. Mix thoroughly by flicking the tube, and then spin down.

Table 15 Round One in vitro transcription reaction mix

Complete 2nd Strand Synthesis Mix			6 reaction Master Mix with 10% overage (µL)
Component	**Amount ()**	**Vial #**	
IVT Buffer	2 µL	Blue-1	13.2 µL
IVT Master Mix	6 µL	Blue-2	39.6 µL
IVT Enzyme Mix	2 µL	Blue-3	13.2 µL
Enhancer	2 µL	Yellow-E	13.2 µL
Total per sample	12 µL	—	79.2 µL

4. Incubate at 42°C for 3 hours (Optional: You can use four-hour incubation for additional aRNA yield). Chill the sample(s) to 4–8°C.

Note: At this point in the protocol, you may hold the reaction mixture at 4–8°C in the thermal cycler overnight.

5. Move the samples directly to a 4–8°C block.
6. Add 1 µL DNase Mix (Blue-4). Mix thoroughly, and spin down.

   Note: DNase Mix must be thoroughly mixed and held at 4 °C until used.
7. Incubate at 37°C for 15 minutes.
8. Chill the sample(s) to 4–8°C. Proceed immediately to aRNA purification.

   Note: RNA may be adversely affected if not purified immediately after DNase treatment.

Round One: aRNA Purification

Note: RNA Binding Buffer (Blue-RB) must be at room temperature and thoroughly mixed before use. A precipitate may form during long-term storage. Dissolve precipitate prior to use by mixing. If necessary, warm the RB vial to re-dissolve.

1. Add 250 µL of RNA Binding Buffer (Blue-RB) to a new purification column and incubate for 5 minutes at room temperature.
2. Centrifuge at 16,000 x g for 1 minute.
3. Add 120 µL of RNA Binding Buffer to the IVT reaction sample and mix thoroughly.
4. Pipet the entire volume into the purification column.
5. To bind aRNA, centrifuge at 100 x g (or lowest speed setting available) for 2 minutes, immediately followed by a centrifugation at 10,000 x g for 1 minute to remove flow-through.
6. Add 200 µL of RNA Wash Buffer (Blue-RW) to the purification column and centrifuge at 10,000 x g for 1 minute.
7. Add 200 µL of fresh RNA Wash Buffer to the purification column, and centrifuge at 16,000 x g for 2 minutes.
8. Check the purification column for any residual wash buffer. If any wash buffer remains, re-centrifuge at 16,000 x g for 1 minute.
9. Discard the collection tube and flow-through.

   IMPORTANT! Avoid splashing flow-through in the collection tube onto the purification column. If flow-through waste liquid wets the outside of the purification column, re-centrifuge the column at 16,000 x g to remove the liquid.
10. Place the purification column into a new 0.5-mL microcentrifuge tube provided in the kit and carefully add RNA Elution Buffer (Blue-RE) directly to the center of the purification column membrane. Add one of the following volumes:
    - 30 µL if stopping with one round of amplification.
      or
    - 12 µL if going on to a second round.
11. Gently touch the tip of the pipette to the surface of the membrane while dispensing RE to ensure maximum absorption of RE into the membrane. Gently tap the purification column to distribute the buffer, if necessary.
12. Incubate at room temperature for 1 minute.
13. Place each column-tube assembly into the centrifuge rotor with the 0.5-mL tube cap trailing the tube.
14. Centrifuge at 1000 x g for 1 minute, and immediately centrifuge at 16,000 x g for 1 minute. Discard the purification column and retain the elution containing the aRNA.

IMPORTANT! Tubes must be properly oriented in the rotor during elution. To avoid potential breakage of the microcentrifuge tube cap during centrifugation, insert the purification tube/0.5-mL tube assembly into a lidless 1.7/2.0-mL tube. Insert this assembly into adjacent rotor holes (see Figure 2 on page 52). Rest the tube cap against the tube immediately clockwise to it. Place an empty, lidless 1.7/2.0-mL tube into the rotor hole adjacent in the clockwise direction to the last assembly.

The purified aRNA is ready for use in a labeling reaction, qRT-PCR, or for use in a second round of amplification as described in the following section of this User Guide. Purified aRNA may be stored at –70°C. Immediately proceed to Round Two or store the purified aRNA at -70°C overnight.

END OF ROUND ONE

RiboAmp® PLUS Round Two

Protocol for performing a second round of amplification

You can perform a second round of amplification to increase the yield of the amplification, if necessary. In second round amplification, purified aRNA product from Round One is used to produce double-stranded cDNA, which in turn is used as template for an *in vitro* transcription reaction.

IMPORTANT! Do not use the following second-round amplification protocol without first performing a first round of amplification.

There are two significant differences between the first-round and second-round amplification protocols.

- Since Primer A is a component of 2nd Strand Synthesis, and Primer B is a component of 1st Strand Synthesis, reaction temperatures and incubation intervals are different.
- The second-round amplification protocol does not make use of 1st Strand Nuclease Mix.

Note: The aRNA product produced after the second round of amplification is somewhat shorter than that formed from one round. Typically, the bulk of the aRNA visualized through gel electrophoresis will range from under 200 to over 600 bases.

Round Two: 1st strand cDNA synthesis

1. Thaw samples from round one at 4–8 °C if necessary. Place samples on a 4–8°C block.
2. Thaw Primer B (Grey-B), thoroughly mix, spin down, and place on a 4–8°C block.
3. Into eluted aRNA product from Round One, add 1.0 µL of Primer B, mix thoroughly by flicking the tube, and spin down.
4. Incubate the microcentrifuge tube at 65°C for 5 minutes then chill the samples to 4–8°C for 1 minute.
5. Spin down the contents and place on 4–8°C block before proceeding to next step.
6. Place 1st Strand Synthesis components (Red-1 and Red-2) at 4–8°C. 1st Strand Master Mix must be thawed, thoroughly mixed with all solids dissolved, and maintained at 4–8°C until used.

Note: 1st Strand Enzyme Mix does not require thawing and can be placed directly at 4–8°C. Mix enzyme thoroughly by inverting several times. Spin briefly.

7. Add 1st Strand Synthesis components in the order listed in Table 16 on page 33.

   Note: If you are performing several amplifications, you may wish to prepare a Complete 1st Strand Synthesis Mix based on the table below, and add 9.0 µL Complete 1st Strand Synthesis Mix to each sample. Mix thoroughly by flicking the tube, and spin down. *Do not vortex*.

Table 16 Round Two 1st-strand cDNA synthesis reaction mix

Complete 1nd Strand Synthesis Mix			6 reaction Master Mix with 10% overage
Component	**Amount**	**Vial #**	
1st Strand Master Mix	7 µL	Red-1	46.2 µL
1st Strand Enzyme Mix	2 µL	Red-2	13.2 µL
Total per sample	9 µL	—	59.4 µL

8. Incubate the sample(s) at 25°C for 10 minutes then at 37°C for 45 minutes.
9. Chill the sample(s) to 4–8°C for at least 1 minute.

Note: It is safe to stop at this point in the protocol. You can store the sample overnight at -20°C.

IMPORTANT! Place the components back onto the cold block or refreeze immediately after dispensing the reagent. Do not leave reagents at room temperature for any extended period of time.

Round Two: 2nd strand cDNA synthesis

1. Place sample on a 4–8°C block and allow to thaw, if frozen (at 4–8°C).
2. Thaw Primer A (Gray-A), thoroughly mix, spin down, and place on a 4–8°C block.
3. Add 1.0 µL of Primer A to the sample. Mix thoroughly by flicking the tube, and spin down.
4. Incubate the sample at 95°C for 2 minutes, then cool sample to 4–8°C for at least 1 minute. Hold the sample at 4–8°C until ready to proceed.
5. Spin down the contents, and place on 4–8°C block before proceeding to the next step.
6. Thaw the 2nd Strand Master Mix (Green-1) at 4–8°C (cold block). Thoroughly mix and spin 2nd Strand Master Mix.

   Note: 2nd Strand Enzyme Mix (Green-2) does not require thawing. Mix enzyme thoroughly by inverting several times, spin briefly, and place at 4–8°C.

7. Add 2nd Strand Synthesis components separately in the order listed in Table 17.

Note: If you are performing several amplifications, you may want to prepare a Complete 2nd Strand Synthesis Mix based on Table 17, and add 30 µL Complete 2nd Strand Synthesis Mix to each sample. Mix thoroughly by flicking the tube, and spin down.

Table 17 Round Two 2nd-strand cDNA synthesis reaction mix

Complete 2nd Strand Synthesis Mix			6 reaction Master Mix with 10% overage (µL)
Component	Amount (µL)	Vial #	
2nd-Strand Master Mix	29 µL	Green-1	191.4 µL
2nd-Strand Enzyme Mix	1 µL	Green-2	6.6 µL
Total per sample	30 µL	—	198 µL

8. Incubate the sample(s) as follows:
   - 37°C 15 minutes
   - 70°C 5 minutes
   - 4–8°C Hold until ready to proceed (up to a maximum of 30 minutes)

IMPORTANT! Place components back onto the cold block or refreeze immediately after dispensing the reagent. Do not leave reagents at room temperature.

Round Two: cDNA purification

Note: RNA Binding Buffer (Red-RB) must be at room temperature and thoroughly mixed before use. A precipitate may form during long-term storage. Dissolve precipitate prior to use by mixing. If necessary, warm the RB vial to re-dissolve.

1. Add 250 µL of DNA Binding Buffer (Red-DB) to a new purification column seated in the collection tube provided. Incubate for 5 minutes at room temperature. Centrifuge at 16,000 x g for 1 minute.
2. Add 200 µL of DNA Binding Buffer to the 2nd Strand Synthesis sample tube, mix well, and pipette the entire volume into the purification column.
3. To bind cDNA, centrifuge at 100 x g (or lowest speed setting available) for 2 minutes, then immediately centrifuge at 10,000 x g for 1 minute to remove flow-through.
4. Add 250 µL of DNA Wash Buffer (Red-DW) to the column and centrifuge at 16,000 x g for 2 minutes. Check the purification column for any residual wash buffer. If any wash buffer remains, re-centrifuge at 16,000 x g for 1 minute.
5. Discard the collection tube and flow-through.

   Note: Avoid splashing flow-through in the collection tube onto the purification column. If flow-through waste liquid wets the outside of the purification column, re-centrifuge the column at 16,000 x g to remove the liquid.
6. Place the column into the provided 0.5-mL microcentrifuge tube and carefully add 11 µL of DNA Elution Buffer (Red-DE) onto the center of the purification column membrane. Gently touch the tip of the pipette to the surface of the membrane while dispensing DE to ensure maximum absorption of DE into the membrane. Gently tap the purification column to distribute the buffer, if necessary.
7. Incubate for one minute at room temperature.
8. Place each column-tube assembly into the 2-mL support tube in the rotor with the 0.5-mL tube cap trailing the tube.
9. Centrifuge at 1000 x g for 1 minute, and then immediately centrifuge by 16,000 x g for 1 minute. Discard the column and retain the elution containing the cDNA.

IMPORTANT! To avoid potential breakage of the microcentrifuge tube cap during centrifugation, insert the purification tube/0.5-mL tube assembly into a lidless 1.7/2.0-mL tube. Insert this assembly into adjacent rotor holes as illustrated in Appendix D, "Centrifuge Information". Rest the tube cap against the tube immediately clockwise to it. Place an empty, lidless 1.7/2.0-mL tube into the rotor hole adjacent in the clockwise direction to the last assembly.

Note: It is safe to stop at this point in the protocol. You can store the sample overnight at -20°C.

IMPORTANT! 1.5 Round Users Using Alternate IVT Labeling Procedures: *Stop Here.*

1.5 Round users (KIT0526) using alternative IVT labeling (such as Affymetrix labeling kit 900449), *stop here*, and proceed to Appendix C, "Generating Labeled aRNA Using Alternative IVT Kits".

Note: Life Technologies recommends the Turbo Labeling™ microarray kit (Biotin - KIT0608, CY®3 - KIT0609, CY®5 - KIT0610) for higher yields and longer aRNA fragments. The Turbo Labeling™ kit allows for a more efficient labeling process leading to a better representation of the mRNA transcript and higher present calls.

Round Two: In vitro transcription

IVT reaction components must be thawed, thoroughly mixed with all solids dissolved, and brought to room temperature just before use.

1. Thaw IVT Buffer (Blue-1), Master Mix (Blue-2) and Enhancer (Yellow-E) to room temperature and thoroughly mix to dissolve all solids.
2. IVT Enzyme Mix (Blue-3) does not require thawing and can be put in directly at 4–8 °C. Mix enzyme thoroughly by inverting several times. Spin briefly.
3. Add IVT components in the order listed in Table 18.

Note: If you are performing several amplifications, you may want to prepare a Complete IVT Reaction Mix according to Table 18, and add 12 µL Complete IVT Reaction Mix to each sample. Mix thoroughly by flicking the tube, and spin down.

Table 18 Round two in-vitro transcription reaction mix

Complete IVT Mix			6 reaction Master Mix with 10% overage
Component	**Amount**	**Vial #**	
IVT Buffer	2 µL	Blue-1	13.2 µL
IVT Master Mix	6 µL	Blue-2*	39.6 µL
IVT Enzyme Mix	2 µL	Blue-3	13.2 µL
Enhancer	2 µL	Yellow-E	13.2 µL
Total per sample	12 µL		79.2 µL

Note: If you are doing amino-allyl incorporation, substitute Amino-Allyl IVT Master Mix (teal-AA) here.

4. Incubate at 42°C for 4 hours (*Optional*: If you want additional yield, you can incubate the IVT reaction for up to six hours). Chill the sample(s) to 4–8°C.

Note: It is safe to stop at this point in the protocol. You may hold the reaction mixture at 4–8°C in the thermal cycler overnight.

5. Move the samples directly to a 4–8°C block.
6. Add 1 µL DNase Mix (Blue-4). Mix thoroughly and spin down. Incubate at 37°C for 15 minutes. Chill the sample(s) to 4–8°C. Proceed immediately to aRNA purification.

Note: DNase Mix must be thoroughly mixed and held at 4°C until used. RNA may be adversely affected if not purified immediately after DNase.

Round Two: aRNA purification

1. Add 250 µL of RNA Binding Buffer (Blue-RB) to a new purification column seated in the collection tube provided. Incubate for 5 minutes at room temperature. Centrifuge at 16,000 x g for 1 minute.

   Note: RNA Binding Buffer (Blue-RB) must be at room temperature and thoroughly mixed before use. A precipitate may form during long-term storage. Dissolve precipitate prior to use by mixing. If necessary, warm the RB vial to re-dissolve.

2. Add 120 µL of RNA Binding Buffer to the IVT reaction sample and mix thoroughly. Pipet the entire volume into the purification column.
3. To bind aRNA, centrifuge at 100 x g (or lowest speed setting available) for 2 minutes, and immediately centrifuge at 10,000 x g for 1 minute.
4. Add 200 µL of RNA Wash Buffer (Blue-RW) to the purification column and centrifuge at 10,000 x g for 1 minute.
5. Add 200 µL of fresh RNA Wash Buffer to the purification column, and centrifuge at 16,000 x g for 2 minutes. Check the column for any residual wash buffer. If any wash buffer remains, re-centrifuge at 16,000 x g for 1 minute.
6. Discard the collection tube and flow-through.

   IMPORTANT! Avoid splashing flow-through in the collection tube onto the purification column. If flow-through waste liquid wets the outside of the purification column, re-centrifuge the column at 16,000 x g to remove the liquid.

7. Place the purification column into a new 0.5-mL microcentrifuge tube provided in the Kit and carefully add 30 µL of RNA Elution Buffer (Blue-RE) directly to the center of the purification column membrane.

   Note: Gently touch the tip of the pipette to the surface of the membrane while dispensing RE to ensure maximum absorption of RE into the membrane. Gently tap the purification column to distribute the buffer, if necessary.

8. Incubate for one minute at room temperature.
9. Place each column-tube assembly into the 2-mL support tube in the rotor with the 0.5-mL tube cap trailing the tube.
10. Centrifuge at 1000 x g for 1 minute, followed immediately by 16,000 x g for 1 minute. Discard the column and retain the elution containing the aRNA.

IMPORTANT! To avoid potential breakage of the microcentrifuge tube cap during centrifugation, insert the purification tube/0.5-mL tube assembly into a lidless 1.7/2.0-mL tube. Insert this assembly into adjacent rotor holes. Rest the tube cap against the tube immediately clockwise to it. Place an empty, lidless 1.7/2.0-mL tube into the rotor hole adjacent in the clockwise direction to the last assembly.

11. Measure the O.D. of the product at A_{260} and A_{280}.

12. Analyze the aRNA using the Agilent Bioanalyzer or by gel electrophoresis.

The purified aRNA is ready for use in a labeling reaction with the Turbo Labeling™ kit for microarrays. You can store purified aRNA at –70°C.

6

Troubleshooting

Chapter contents:

- Amplification yield is poor 39
- Low molecular weight product appears on a gel 41

Amplification yield is poor

The table below lists possible reasons why the amplification yield is poor, and provides suggestions for correcting the problem.

Table 19 Suggestions for correcting low amplification yield

Cause	Suggestion
Starting RNA sample quality varies	If you observe low yields with different RNA samples, run an amplification control using the Control RNA provided in the RiboAmp® PLUS Kit to verify kit functionality.
Starting RNA sample quality has been compromised.	The greatest factor affecting amplification efficiency is the integrity of the RNA used in the RiboAmp® PLUS amplification process. Suspend RNA in nuclease-free water prior to amplification. Avoid using organic solvents such as phenol in RNA isolation procedures. Trace amounts of organic solvents that carry over into amplification reactions will impair the amplification process. If input RNA is from cells obtained by LCM, use specialized LCM sample preparation protocols designed to preserve RNA. Arcturus® HistoGene® and PicoPure® Kits are optimized to preserve the integrity of RNA and maximize recovery. You can use Quantitative Real-Time PCR of 1st Strand Synthesis product to verify the quality and quantity of the RNA input.
There is no RNA in the input sample.	Run a control RNA sample with a known quantity of RNA to ensure that amplification is successful.
Reagent concentrations in reaction mixtures are incorrect due to inadequate thawing or mixing.	Ensure all reagents are completely thawed, mixed, and all solids dissolved prior to use.

Cause	Suggestion
Reagent concentrations in the reaction mixtures are incorrect due to inadequate reaction volume collection in the reaction tube.	Thoroughly thaw and mix all reagents prior to dispensing. Ensure all reagents are dispensed at proper volumes. Briefly spin down the reaction mix prior to incubation to ensure all reagents are collected in the reaction volume and the reaction mix has the proper concentrations of reagents.
Reagent concentrations in reaction mixtures are incorrect due to evaporative condensation onto the wall of the reaction tube during incubation.	Briefly spin down the sample following incubation steps to maintain proper volumes and concentrations of reagents and ensure that all nucleic acid templates are mixed with reaction components. Use a thermal cycler with a heated lid.
Incubation temperatures are incorrect.	Verify the accuracy of all incubation temperatures. If you are using a thermal cycler, make sure that the programmed temperatures read correctly and the instrument has been calibrated to establish and maintain accurate temperature settings.
RNA yield is diminished during column purification.	Verify centrifugal force used during nucleic acid purification. Improper binding, washing, and elution centrifugal forces can decrease the recovery of nucleic acid from the purification column. Microcentrifuges should be calibrated to deliver the correct centrifugal force.
Message content is low within the total RNA being used in your study.	Check amplification efficiency using control RNA. Use higher RNA inputs to compensate for lower message content.

Low molecular weight product appears on a gel

Occasionally, a predominant band below the expected aRNA smear will appear on a gel. This band will lead to improper estimation of yield and may result in high backgrounds on microarrays. The RiboAmp® PLUS Kit components are formulated and tested to avoid the synthesis of this material, but if it does occur, refer to Table 20

The table below lists possible reasons why low molecular weight material is present, and provides suggestions for correcting the problem.

Table 20 Suggestions for correcting low molecular weight in gel

Cause	Suggestion
Quality of the starting RNA is inadequate.	Poor RNA quality can lead to the formation of the reaction artifact, visible as a low molecular weight band. Check the quality of your input RNA. One approach is to utilize the Agilent Lab-on-a-Chip System with an RNA LabChip® Kit. For additional recommendations to check for the quality of the input RNA, contact Technical Support at +1-800-831-6844.
Concentrations of Primer 1, Primer 2, Primer 3, or 1st Strand Nuclease Mix are incorrect due to inadequate thawing or dispensing.	Thaw and thoroughly mix each reagent vial prior to dispensing. If incompletely thawed and mixed, the concentrations of these reagents may not be dispensed at optimal concentrations for the reaction. Ensure that all pipettes are properly calibrated to dispense correct volumes.
Concentrations of Primer 1, Primer 2, Primer 3, or 1st Strand Nuclease Mix are incorrect due to inadequate mixing or reaction volume collection inside the reaction tube.	Thoroughly mix and spin down the sample after adding the primers or 1st Strand Nuclease Mix into the reaction mix and prior to incubation. This ensures the correct concentration of primers or nuclease in each respective reaction mix.
Input RNA was not isolated using the PicoPure® RNA Isolation Kit and no nucleic acid carrier was added.	Low molecular weight material may result from lack of RNA and carrier. Using the PicoPure® RNA Isolation Kit is recommended to prepare samples that contain carrier.

Amino-allyl aRNA Labeling Protocol

This appendix covers:

- Appropriate reagents 43
- Labeling reaction 43
- aRNA purification 44

Appropriate reagents

This protocol is intended for use with amino-allyl modified aRNA that was generated using the optional Amino-Allyl IVT components of RA7010 and RA7012. The table below lists appropriate reagents.

Table 21 Reagents appropriate for Amino-allyl aRNA labeling

Reagent	Maker	Catalog Number
CY®3 mono reactive dye	Amersham	PA23001
CY®5 mono reactive dye	Amersham	PA25001
Alexa Fluor® 647 reactive dye decapacks for microarrays	Molecular Probes	A-32756
Alexa Fluor® 555 reactive dye decapacks for microarrays	Molecular Probes	A-32757

Labeling reaction

Re-suspend 1 mg monoreactive dye in 51 µL of DMSO (Teal-DMSO). Save unused vials in the dark at 2–6°C.

1. Take 15 µg of amino-allyl aRNA in 7.5 µL of nuclease free water.

   Note: Maintain sample on a cold block.

2. Add 2.5 µL of Labeling Buffer (Teal-LB) to the sample.
3. Add 10 µL of the re-suspended dye into 10 µL of the sample.
4. Mix thoroughly by flicking the tube. Spin down briefly.
5. Incubate at room temperature in the dark for 1 hour.
6. Proceed directly to purification of labeled aRNA.

aRNA purification

1. Pre-treat column by adding 250 µl of RNA Binding Buffer (Blue-RB) to a new purification column. Incubate the column at room temperature for 5 minutes. Centrifuge at 16,000 x g for 1 minute.
2. Add 225 µl of RNA Binding Buffer to the transcript labeling reaction sample and mix thoroughly. Pipette the entire sample volume into the purification column.
3. Centrifuge at 100 *x g* (or lowest speed setting available) for 2 minutes, and immediately centrifuge at 10,000 x g for 1 minute.

   Note: To obtain 15 µg of aRNA in 7.5 µl, you can dry down 15 µg of aRNA and re-suspend in 7.5 µl of nuclease free water, or concentrate the aRNA to 2 µg /µl and use 7.5 µl of the sample.

   - Do not use re-suspended dye that is over 2 days old. DMSO is hygroscopic. Store tightly capped.
   - Do not allow the samples to incubate longer than 1 hour.
   - Use reagents supplied in the Labeling Purification Reagents box.
4. Discard flow-through. Place the column into the same collection tube.
5. Add 250 µl of RNA Wash Buffer (Teal-RW) to the purification column and centrifuge at 10,000 x g for 1 minute.
6. Repeat Step 5.
7. Add 250 µl of fresh RNA Wash Buffer to the column and centrifuge at 16,000 x g (full speed) for 2 minutes. Check the purification column for any residual wash buffer. If any wash buffer remains, re-centrifuge at 16,000 x g for 1 minute.
8. Discard the collection tube and flow-through.
9. Place the purification column into a new 0.5 mL microcentrifuge tube provided in the kit and carefully add 50 µl of RNA elution Buffer (Teal-RE) directly onto the center of the purification column membrane. Gently touch the tip of the pipette to the surface of the membrane while dispensing RE to ensure maximum absorption of RE into the membrane. Gently tap the purification column to distribute the buffer if necessary.
10. Incubate at room temperature for 1 minute.
11. Place the assembly into the 2 mL support tube in the rotor with the 0.5 mL tube cap trailing the tube.
12. Centrifuge at 1000 x g for 1 minute, immediately followed by 16,000 x g for 1 minute. Discard the purification column and retain the elution containing the labeled aRNA.
13. Measure the O.D. of the product at A_{260}, A_{280}, and A_{550}/A_{650} to determine the yield and frequency of incorporation (FOI) by making a dilution of 1:10 (5 µl sample + 45 µl nuclease free water).
14. Store any remaining samples at –70°C until ready for hybridization.

B aRNA Analysis

This appendix covers:

- Determining RNA yield and purity 45
- Assessing input RNA sample quality 45
- Assessing total RNA and aRNA quality 46
- Analyzing aRNA by agarose gel electrophoresis 47

Determining RNA yield and purity

aRNA quantitation by ultraviolet light absorbance is the simplest approach to determining amplification yield. An absorbance reading at 260 nm (A_{260}) using a spectrophotometer is taken on a diluted aliquot of aRNA. Typically, a 1:25 to 1:50 dilution of aRNA in nuclease free water is sufficient.

For single-stranded RNA, a measurement of A_{260} = 1.0 corresponds to 40 µg/mL. The yield can by calculated by:

(A_{260}) *(dilution factor) (40) = µg/mL RNA*

Measuring A_{280} and calculating the A_{260}/A_{280} ratio indicates the purity of the RNA sample. An A_{260}/A_{280} ratio between 2.0 and 2.6 indicates very pure aRNA.

Assessing input RNA sample quality

This section provides instructions for assessing input RNA sample quality by quantitative Real-Time PCR.

Although gel electrophoresis is a common approach to assessing RNA quality, it is not possible to run a gel on the small quantities of input RNA used for amplification with the RiboAmp® PLUS Kit. Therefore, you may want to assess the input RNA quality after 1st Strand Synthesis using quantitative Real-Time PCR (qRT-PCR). You can use the following guidelines.

To prepare:

- Wipe all surfaces and equipment with RNase decontamination solution.
- Use RNase-free solutions and plastic ware.
- Wear disposable gloves.

Perform amplification following the RiboAmp® PLUS Kit protocol.

1. During Round One 1st Strand Synthesis, remove 2 µL of the sample.
2. Pipette into a new 0.5- or 0.2-mL microcentrifuge tube.

3. Add 8 µL of nuclease-free water. This is the diluted cDNA template.
4. Mix the sample well. Spin down, and store on ice until ready to use.
5. Proceed according to protocols and Instruction Manuals for the qRT-PCR system utilized. Use 2 µL of diluted cDNA template (from step 4). Refer to the qRT-PCR system manual for details concerning interpretation of data.

Assessing total RNA and aRNA quality

This section covers assessing Total RNA and aRNA quality using the Agilent Lab-on-a-Chip system.

The Agilent Lab-on-a-Chip system provides a fast and effective approach to assessing the integrity of an aRNA sample. The system requires very small quantity of sample. Refer to the Agilent 2100 Bioanalyzer and RNA LabChip® Kit Instruction Manuals for details.

Required equipment and materials

This equipment and these materials are required for this procedure:

- Agilent 2100 Bioanalyzer System (Agilent)
- RNA 6000 Nano Assay Kit (Agilent)
- Ice or cold block (4-8°C)
- Spectrophotometer

Before you begin, refer to the instruction manual for the RNA 6000 Nano Assay Kit. Prepare necessary reagents and supplies as required by the kit.

To prepare:

- Wipe all surfaces and equipment with RNase decontamination solution.
- Use RNase-free solutions and plastic ware.
- Wear disposable gloves.

Suggested protocol

1. Determine the concentration of the aRNA generated through RiboAmp® PLUS by UV spectrophotometry.
2. Based on the optical density reading, prepare a dilution of the sample to a concentration of 200–300 ng/µL.

   Note: Store the sample on ice or in a cold block until ready to load on to the RNA chip.
3. Follow the RNA 6000 Nano Assay Kit protocol, loading 1 µL of the prepared sample dilution (from step 2).

For details of data interpretation refer to the bioanalyzer instruction manual. The aRNA appears on the bioanalyzer as a single, broad peak. The size of the aRNA ranges in length from 200 to 2000 bases.

Analyzing aRNA by agarose gel electrophoresis

Analyzing aRNA using agarose gel electrophoresis is one method to visualize the RNA profile and relative quantity after amplification. You can use standard protocols for agarose gel electrophoresis. The following protocol uses commercially available reagents.

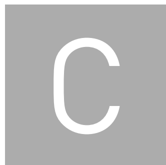
Required materials

These materials are required for this procedure:

- 1.25% Agarose Portrait Gel or 1.25 Agarose Medium Gel (EmbiTec cat. # GE-6010 or GE-6030)
- 10X RNA MOPS Running Buffer (EmbiTec cat. # EC-1020)
- 2X Gel Loading Buffer (various)
- RNA Ladder (various)
- SYBR® Gold Nucleic Acid Gel Stain (Molecular Probes cat. # S-11494) or Ethidium Bromide Stain
- Nuclease-free Water

Suggested protocol

1. Determine the concentration of the aRNA by UV absorbance with a spectrophotometer.
2. Dilute the aRNA sample(s) with nuclease-free water. Each gel well can be loaded with 1 – 3 µg of aRNA.
3. Prepare aRNA gel sample by mixing 6 µL of diluted aRNA with 6 µL of 2X Gel Loading Buffer.
4. Incubate for 3–5 minutes at 65°C. Cool on ice.
5. Prepare 1X RNA MOPS Running Buffer and fill gel electrophoresis unit. Place agarose gel into the unit.
6. Load 12 µL of sample per well of the agarose gel. Include RNA Ladder in one or more lanes.
7. Electrophorese at 5–7 volts per centimeter for 30 minutes.
8. Stain the gel with SYBR Gold Nucleic Acid Gel Stain for 30 minutes or according to the protocol supplied with the reagent. Alternatively, stain with Ethidium Bromide (0.5–1.0 µg/mL).
9. Visualize the gel on a UV transilluminator. The size of the aRNA ranges from 200 to 2000 bases in length.



Generating Labeled aRNA Using Alternative IVT Kits

This appendix covers:

- Overview of substitution process. 49
- Antisense RNA purification 49

Overview of substitution process

This appendix explains how to use the RiboAmp® PLUS Kits with alternative IVT labeling (such as Affymetrix labeling kit 900449) to yield suitable RNA sample for hybridizing to GeneChip Probe Arrays.

You substitute these kit reagents and protocol during the second IVT reaction of the RiboAmp® PLUS Kit protocol. You subsequently purify the Labeled aRNA with the RiboAmp® PLUS Kit and MiraCol™ Purification Columns as described below.

1. Perform Round One of amplification according to the RiboAmp® PLUS Amplification Kit protocol starting from the recommended input for the kit.

   Note: Due to IVT efficiency, we do not recommend using the minimum input amounts with an alternative labeling kit.

2. Perform Round Two of amplification through cDNA Purification according to the Kit protocol.

3. Perform RNA transcript labeling according to the protocol of the IVT labeling kit using the sample (from step 2 above) as the cDNA template. Adjust the final volume of the cDNA sample, as necessary.

Antisense RNA purification

1. Add 250 µL of RNA Binding Buffer (Blue-RB) to a new purification column and incubate for 5 minutes at room temperature. Centrifuge at 16,000 x g for 1 minute.

2. Add 200 µL of RNA Binding Buffer to the Transcript Labeling Reaction sample and mix thoroughly. Pipet the entire sample volume into the purification column.

3. Centrifuge at 100 x g (or lowest speed setting available) for 2 minutes, and immediately centrifuge at 10,000 x g for 1 minute.

4. Add 200 µL of RNA Wash Buffer (Blue-RW) to the purification column and centrifuge at 10,000 x g for 1 minute.

   Note: RNA Binding Buffer (Blue-RB) must be at room temperature and thoroughly mixed before use. A precipitate may form during long-term storage. Dissolve precipitate by mixing. If necessary, warm the RB vial to redissolve.

5. Add 200 μL of fresh RNA Wash Buffer to the column and centrifuge at 16,000 x g for 2 minutes. Check the purification column for any residual wash buffer. If any wash buffer remains, re-centrifuge at 16,000 x g for 1 minute.
6. Discard the collection tube and flow-through.
7. Place the purification column into a new 0.5-mL microcentrifuge tube provided in the kit and carefully add 30 μL of RNA Elution Buffer (Blue-RE) directly onto the center of the purification column membrane.

   Note: Gently touch the tip of the pipette to the surface of the membrane while dispensing RE to ensure maximum absorption of RE into the membrane. Gently tap the purification column to distribute the buffer, if necessary.
8. Incubate at room temperature for 1 minute.
9. Place the assembly into the 2-mL support tube in the rotor with the 0.5-mL tube cap trailing the tube.
10. Centrifuge at 1000 x g for 1 minute, and immediately centrifuge at 16,000 x g for 1 minute. Discard the purification column and retain the elution containing the labeled aRNA.
11. Measure the O.D. of the product at A_{260} and A_{280} to determine the yield of labeled aRNA. Perform electrophoretic analysis, if necessary.
12. Proceed to protocols for microarray hybridization.

    IMPORTANT! Avoid splashing flow-through in the collection tube onto the column. If flow-through waste liquid wets the outside of the purification column, re-centrifuge the column at 16,000 x g to remove liquid.

Note: Tubes must be properly oriented in the rotor during elution.

Centrifuge Information

This appendix covers:

- Centrifugal force 51
- Centrifuge rotation 52

Centrifugal force

The table below shows corresponding centrifugal forces (g) for selected rotations per minute (rpm) when you are working with the tabletop microcentrifuge Eppendorf® 5415.

Table 22 Centrifugal forces for selected rotations

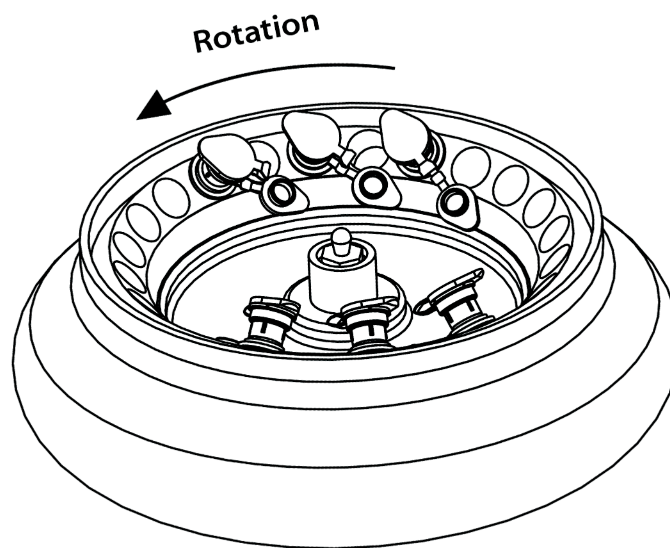
Rotations Per Minute	Centrifugal Force
14,000 rpm	13,000 g
12,000 rpm	10,000 g
10,000 rpm	7000 g
8000 rpm	4500 g
5500 rpm	2200 g
5000 rpm	2000 g

Centrifuge rotation

The graphic below shows the centrifuge.

IMPORTANT! IMPORTANT! To avoid potential breakage of the microcentrifuge tube cap during centrifugation, insert the purification tube/0.5-mL tube assembly into a lidless 1.7/2.0 mL tube. Insert this assembly into adjacent rotor holes as illustrated below. Rest the tube cap against the tube immediately clockwise to it. Place an empty, lidless 1.7/2.0 mL tube into the rotor hole adjacent in the clockwise direction to the last assembly.

Figure 2 Centrifuge drawing



Related Reagent Kits

This appendix covers:

- HistoGene® LCM Frozen Section Staining Kit 53
- HistoGene® LCM Immunofluorescence Staining Kit 53
- PicoPure® RNA Isolation Kit 54
- PicoPure® DNA Extraction Kit 54
- Paradise® PLUS FFPE Kits 54
- Paradise® PLUS FFPE WT-RT Kit 54
- RiboAmp® PLUS RNA Amplification Kits 55
- Turbo Labeling™ Kits 55

Note: Only the most frequently used kits are listed here. Additional kit configurations are available depending on individual research needs. For more information, go to: **www.appliedbiosystems.com**

HistoGene® LCM Frozen Section Staining Kit

The HistoGene® LCM Frozen Section Staining Kit is used to process tissue sections for LCM in order to maximize the quality and yield of RNA from the LCM cells. The kit comes with all dehydration and staining reagents, disposable staining jars, specially treated slides, and a detailed protocol and troubleshooting guide.

KIT0401: 72 slides

HistoGene® LCM Immunofluorescence Staining Kit

The HistoGene® LCM Immunofluorescence Staining Kit is designed to enable retrieval of high-quality RNA from immunofluorescently stained frozen tissue. It enables convenient and reliable staining, dehydration, and LCM of tissue sections. The kit's protocols are streamlined and optimized for efficient LCM capture while maintaining RNA quality for downstream applications that require intact RNA, such as microarray analysis and RTPCR.

KIT0420: 32 slides

PicoPure® RNA Isolation Kit

The PicoPure® RNA Isolation Kit is used for the extraction and isolation of total RNA from small samples, particularly LCM cells. The PicoPure® RNA Kit comes with optimized buffers, MiraCol™ Purification Columns and an easy-to-use protocol to maximize recovery of high-quality total cellular RNA, ready for amplification with the RiboAmp® Plus RNA Amplification Kits.

KIT0204: 40 isolations

PicoPure® DNA Extraction Kit

The PicoPure® DNA Extraction Kit is optimized to maximize the recovery of genomic DNA from 10 or more cells captured by LCM. The kit comes with reagents and protocols tested to ensure complete extraction of DNA from LCM samples prepared with any standard tissue preparation procedure. DNA prepared using the kit is PCR-ready and needs no additional purification to perform amplification.

KIT0103: 150 HS cap extractions, 30 Macro cap extractions, or 10 tissue scrapes

Paradise® PLUS FFPE Kits

The Paradise® PLUS Reagent System is designed to enable gene expression studies using formalin-fixed paraffin-embedded (FFPE) tissue samples. Components include sample preparation and staining reagents, RNA extraction and isolation reagents, RNA amplification reagents and a comprehensive user guide.

KIT0312: 12 samples

KIT0312B: 12 samples with biotin labeling

KIT0312C: 12 samples with CY®3 labeling

KIT0312D: 12 samples with CY®5 labeling

Paradise® PLUS FFPE WT-RT Kit

The Paradise® PLUS Whole Transcript Reverse Transcription (WT-RT) Reagent System Kit enables QRT-PCR using formalin-fixed, paraffin-embedded (FFPE) tissue samples. The kit was developed specifically to overcome obstacles often associated with formalin-fixed tissue, such as chemical modification and RNA fragmentation.

The kit provides RNA isolation and reverse transcription reagents optimized for use with archived FFPE samples at small sample input amounts, and delivers unparalleled yield, fidelity, and representation. The kit was designed with exon-spanning primers at varying distances from the 3' end of the transcript, and allows the study of splice variants in archived or degraded samples. The Paradise® WT-RT system also allows the use of gene-specific primers for reverse transcription, to suit specific assay requirements.

KIT0315: 12 Samples

RiboAmp® PLUS RNA Amplification Kits

The RiboAmp® PLUS RNA Amplification Kit enables the production of microgram quantities of antisense RNA (aRNA) from as little as picogram amounts of total cellular RNA. Amplified RNA produced using the kit is suitable for labeling and use on expression microarrays. The kit achieves 1,000-to 3,000-fold amplifications in one round of amplification, and up to 1,000,000-fold in two rounds. The kits include microarray labeling options for biotin, fluorescent dyes and amino allyls. Kits are available in two sensitivity options, RiboAmp® Plus (5-40 ng input) and a high-sensitivity version RiboAmp® HS Plus (0.1-to 5-ng input).

KIT0521 RiboAmp® PLUS: (12) 1-round amplifications or (6) 2-round amplifications

Note: This is the User Guide for the kit listed above.

KIT0525 RiboAmp® HS PLUS: (6) 2-round amplifications

Turbo Labeling™ Kits

The Turbo Labeling™ Kits provide a proprietary, non-enzymatic technology for the labeling of unmodified aRNA for gene expression profiling. The unmodified aRNA is labeled post-amplification, thereby avoiding the need to incorporate modified nucleotides. The use of natural nucleotides in the amplification step results in unmodified aRNA with higher yields and longer aRNA fragments, thus providing better representation of the mRNA transcript for downstream analysis.

KIT0608 – Biotin: 12 samples

KIT0609 – CY®3: 12 samples

KIT0610 – CY®5: 12 samples

Chemical Safety

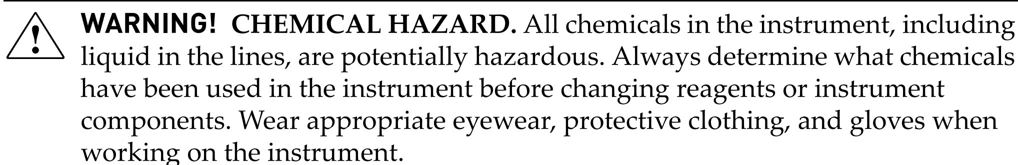
This appendix covers:

- General chemical safety 57
- Safety Data Sheets. 58
- Chemical waste safety 59

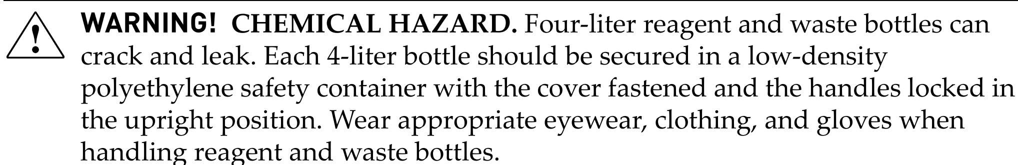
General chemical safety

Chemical hazard warning

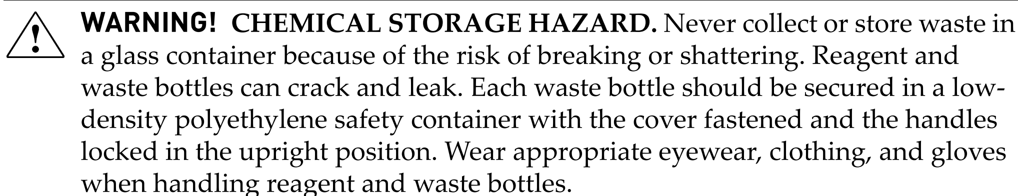
WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.



WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.



WARNING! CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.



WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About SDSs" on page 58.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.

- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Safety Data Sheets

About SDSs

Chemical manufacturers supply current Safety Data Sheets (SDSs) with shipments of hazardous chemicals to new customers. They also provide SDSs with the first shipment of a hazardous chemical to a customer after an SDS has been updated. SDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new SDS packaged with a hazardous chemical, be sure to replace the appropriate SDS in your files.

Obtaining SDSs

The SDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain SDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **SDS**.
2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the SDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Safety Data Sheets and local regulations for handling and disposal.

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

General biohazard

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* found at: **www.cdc.gov/biosafety/publications/index.htm**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:
www.cdc.gov

Documentation and Support

Related documentation

The following documents are available for the Arcturus*XT*™ LCM System and related products:

Document	Part number	Description
	Instruments	
Arcturus*XT*™ LCM System User Guide	0112-0153 Rev. C	Provides a detailed set of instructions for using the Arcturus*XT*™ Instrument.
Arcturus® Troubleshooting Guide	4458770 Rev. A	Provides tips and recommendations for handling problems encountered while using the Arcturus*XT*™ Instrument.
	Reagents	
Arcturus® HistoGene® Frozen Section Staining Kit User Guide	12294-00 Rev. C	Provides instructions for using the Arcturus HistoGene Frozen Section Staining Kit.
Arcturus® HistoGene® Immunofluorescence Staining Kit User Guide	12653-00 Rev. C	Provides instructions for using the Arcturus HistoGene Immunofluorescence Staining Kit.
Arcturus® PicoPure® DNA Extraction Kit User Guide	12637-00 Rev. D	Provides instructions for using the Arcturus PicoPure DNA Extraction Kit.
Arcturus® PicoPure® RNA Isolation Kit User Guide	12682-00ARC Rev. A	Provides instructions for using the Arcturus PicoPure RNA Isolation Kit.
Arcturus® RiboAmp® HS PLUS Kit User Guide	12672-00 Rev. E	Provides instructions for using the Arcturus® RiboAmp® HS PLUS Kit.
Arcturus® Paradise PLUS Reagent System Kit User Guide	12872-00 Rev. D	Provides instructions for using the Arcturus Paradise PLUS Reagent System Kit.
Arcturus® Paradise Whole Transcript RT (WT-RT) Reagent System Kit User Guide	14360-00 Rev. C	Provides instructions for using the Arcturus Paradise PLUS WT-RT Kit.
Arcturus® Turbo Labeling™ Kit User Guide	14827-00 Rev. B	Provides instructions for using the Arcturus Turbo Labeling Kit with Biotin, Cy®3, and Cy®5 dyes.

How to obtain support

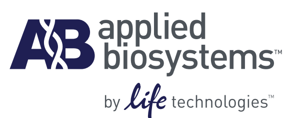
For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, SDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

Part Number 12293-00ARC Rev. B 02/2011



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Technical Resources and Support
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