High-Capacity cDNA Archive Kit

Protocol

For Research Use Only.
Not for use in diagnostic procedures.

Applied Biosystems

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Contents

About the High-Capacity cDNA Archive Kit

Purpose of the Kit

The High-Capacity cDNA Archive Kit contains reagents for reverse transcription (RT) of total RNA to single-stranded cDNA.

Kit Features

Use the kit for the following:

- Quantitative conversion of 0.1 to 10 µg of total RNA to cDNA
- Single-stranded cDNA suitable for quantitative PCR applications
- Single-stranded cDNA suitable for short- or long-term storage

Kit Methods

Use the kit by following one of the following methods:

Method	Description
Automated	The ABI PRISM™ 6700 Automated Nucleic Acid Workstation automates transfer of sample and RT master mix to a cDNA archive plate, places an archive cover on the plate, and heats the plate to perform reverse transcription.
Manual	You manually transfer sample and RT master mix to a cDNA archive plate and place the plate in the GeneAmp® PCR System 9700 or 9600 thermal cycler for reverse transcription.

About This Protocol

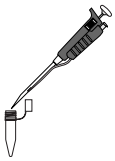
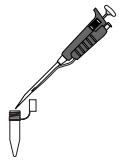
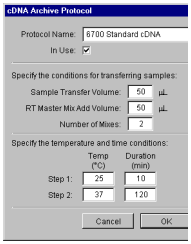
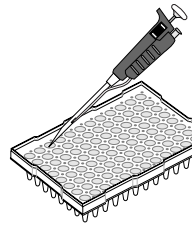
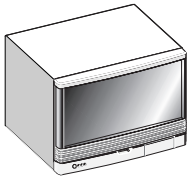
This protocol describes the following:

- Procedures for using the kit
- How to use the kit following the automated method
- How to use the kit following the manual method
- Recommendations for using cDNA archives created using the kit
- Examples of cDNA conversion performance obtained using the kit

Procedure Overview

Procedure Flow

The chart below shows the flow of procedures for the automated and manual methods.

Stage	Automated Method		Manual Method	
1		You prepare RT master mix.		You prepare RT master mix.
2		You set up the 6700 workstation and 6700 system software to run a cDNA archive protocol.		You prepare the cDNA archive reaction plate.
3		The 6700 workstation: ♦ Prepares the cDNA archive reaction plate. ♦ Places an archive cover on the plate. ♦ Heats the plate to perform reverse transcription.	 	You set reverse transcription conditions, load the 9700 or 9600 thermal cycler, and start the run.

Materials and Equipment

Kit Contents

The High-Capacity cDNA Archive Kit (P/N 4322171) contains sufficient quantities to perform 200 reverse transcription reactions with a reaction size of 100 µL.

Component	Quantity
10X RT Buffer, 1.0 mL	2 tubes
10X RT Random Primers, 1.0 mL	2 tubes
25X dNTPs, 1.0 mL	1 tube
MultiScribe™ Reverse Transcriptase, 50 U/µL, 1.0 mL	1 tube

Kit Storage and Stability

Store all components of the kit at –15 to –25 °C.

Equipment Required but Not Supplied

The following table lists the equipment required in addition to the reagents supplied with the High-Capacity cDNA Archive Kit.

Equipment	Source
Equipment for the Automated Method	
ABI PRISM™ 6700 Automated Nucleic Acid Workstation	See your Applied Biosystems sales representative
Equipment for the Manual Method	
GeneAmp® PCR System 9700 thermal cycler	See your Applied Biosystems sales representative
GeneAmp® PCR System 9600 thermal cycler	
Equipment for Both Methods	
Centrifuge with 96-well adapter	Major laboratory supplier (MLS)
Microcentrifuge	MLS
Vortexer	MLS

Materials Required but Not Supplied

The following table lists the materials required in addition to the reagents supplied with the High-Capacity cDNA Archive Kit.

Materials	Source
Materials for the Automated Method	
96-Well Optical Reaction Plate with Barcode	Applied Biosystems (P/N 4306737)
ABI PRISM 6700 Automated Nucleic Acid Workstation User's Manual	Applied Biosystems (P/N 4304309)
Archive Covers	Applied Biosystems (P/N 4306286)
Conductive Pipette Tips, 1000-µL	Applied Biosystems (P/N 4306377)
Conductive Pipette Tips, 200-µL	Applied Biosystems (P/N 4306375)
Reagent Tubes with Caps, 10-mL	Applied Biosystems (P/N 4305932)
Materials for the Manual Method	
Bulkpack MicroAmp® Caps, 12 Caps/Strip	Applied Biosystems (P/N N8011534)
Bulkpack MicroAmp® Caps, 8 Caps/Strip	Applied Biosystems (P/N N8011535)
MicroAmp® Optical 96-Well Reaction Plate	Applied Biosystems (P/N N8010560)
MicroAmp® Optical 96-Well Reaction Plates and Optical Caps	Applied Biosystems (P/N 403012)
MicroAmp® Optical Caps, 8 Caps/Strip	Applied Biosystems (P/N N8010935)
Reagent Tubes with Caps, 10-mL	Applied Biosystems (P/N 4305932)
Materials for Both Methods	
RNase Inhibitor	Applied Biosystems (P/N 8080119)
Nuclease-free H_2O	MLS
Pipette tips, aerosol-resistant	MLS
Pipettors, positive-displacement	MLS

Safety

Documentation User Attention Words

Five user attention words appear in the text of all Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below.

Note Calls attention to useful information.

IMPORTANT Indicates information that is necessary for proper instrument operation.

⚠ CAUTION Indicates a potentially hazardous situation which, 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 which, if not avoided, could result in death or serious injury.

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

Chemical Hazard Warning

⚠ WARNING CHEMICAL HAZARD. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Do not leave chemical containers open. Use only with adequate ventilation.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Site Preparation and Safety Guide

A site preparation and safety guide is a separate document sent to all customers who have purchased an Applied Biosystems instrument. Refer to the guide written for your instrument for information on site preparation, instrument safety, chemical safety, and waste profiles.

About MSDSs

Some of the chemicals used with this instrument may be listed as hazardous by their manufacturer. When hazards exist, warnings are prominently displayed on the labels of all chemicals.

Chemical manufacturers supply a current material safety data sheet (MSDS) before or with shipments of hazardous chemicals to new customers and with the first shipment of a hazardous chemical after an MSDS update. MSDSs provide you with the safety information you need to store, handle, transport and dispose of the chemicals safely.

We strongly recommend that you replace the appropriate MSDS in your files each time you receive a new MSDS packaged with a hazardous chemical.

⚠ WARNING CHEMICAL HAZARD. Be sure to familiarize yourself with the MSDSs before using reagents or solvents.

Ordering MSDSs

You can order free additional copies of MSDSs for chemicals manufactured or distributed by Applied Biosystems using the contact information below.

<table>
<tr><th>To order MSDSs...</th><th>Then...</th></tr>
<tr><td>Over the Internet</td><td>a. Go to our Web site at www.appliedbiosystems.com/techsupp
b. Click MSDSs
<table><tr><th>If you have...</th><th>Then...</th></tr><tr><td>The MSDS document number or the Document on Demand index number</td><td>Enter one of these numbers in the appropriate field on this page.</td></tr><tr><td>The product part number</td><td rowspan="2">Select Click Here, then enter the part number or keyword(s) in the field on this page.</td></tr><tr><td>Keyword(s)</td></tr></table>c. You can open and download a PDF (using Adobe® Acrobat® Reader™) of the document by selecting it, or you can choose to have the document sent to you by fax or e-mail.</td></tr>
<tr><td>By automated telephone service</td><td>Use “To Obtain Documents on Demand” under “Technical Support.”</td></tr>
<tr><td>By telephone in the United States</td><td>Dial 1-800-327-3002, then press 1.</td></tr>
<tr><td>By telephone from Canada</td><td><table><tr><th>To order in...</th><th>Dial 1-800-668-6913 and...</th></tr><tr><td>English</td><td>Press 1, then 2, then 1 again</td></tr><tr><td>French</td><td>Press 2, then 2, then 1</td></tr></table></td></tr>
<tr><td>By telephone from any other country</td><td>See the specific region under “To Contact Technical Support by Telephone or Fax” under “Technical Support.”</td></tr>
</table>

For chemicals not manufactured or distributed by Applied Biosystems, call the chemical manufacturer.

Guidelines for Using the Kit

Recommendations for RNA

For optimal performance of the High-Capacity cDNA Archive Kit, Applied Biosystems recommends using RNA with the following characteristics:

- Greater than 60 µL of sample
- Between 0.002 and 0.2 µg/µL in concentration of RNA
- Less than 0.5% of genomic DNA by weight
- Free of inhibitors of reverse transcription and PCR
- Dissolved in PCR-compatible buffer
- Free of RNase activity

Note If you suspect that the RNA contains RNase activity, add RNase Inhibitor to the reverse transcription reaction at a final concentration of 1.0 U/µL. It is not necessary to add RNase Inhibitor to the reverse transcription reaction if the RNA was purified using the 6700 workstation and Applied Biosystems nucleic acid purification reagents.

- Nondenatured

IMPORTANT It is not necessary to denature the RNA. Denaturation of the RNA may reduce the yield of cDNA for some gene targets.

Reagent and Sample Preparation Guidelines

Follow the guidelines below to ensure optimal performance of the High-Capacity cDNA Archive Kit.

- Use nuclease-free pipette tips and reagents to minimize degradation of the RNA.
- Observe standard laboratory practices when handling RNA.

Automated Method

Procedure Overview

With the automated method, the following events occur:

- You prepare 2X RT master mix
- You set up the 6700 workstation
- The 6700 workstation performs the following:
  - Transfers 50 µL of sample and 50 µL of RT master mix to a cDNA archive plate
  - Places an archive cover on the plate
  - Heats the plate to perform reverse transcription

Preparing Reaction Master Mix

Prepare 2X RT master mix using the kit components.

To prepare 2X RT master mix:

Step	Action
1	Allow the kit components to thaw on ice.
2	Calculate the volume of components needed for the cDNA archive protocol, using the table below. **Component** / **Volume (µL) / Reaction** 10X Reverse Transcription Buffer: 10 25X dNTPs: 4 10X random primers: 10 MultiScribe™ Reverse Transcriptase, 50 U/µL: 5 Nuclease-free H_2O: 21 **Total per Reaction**: 50 **IMPORTANT** Include at least two additional reactions in the calculations to provide excess volume for liquid level sensing by the robotic arm.
3	Pipette the required volumes of components into a 10-mL reagent tube. **⚠ WARNING CHEMICAL HAZARD. 10X RT Buffer** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate eyewear, clothing, and gloves.

To prepare 2X RT master mix: *(continued)*

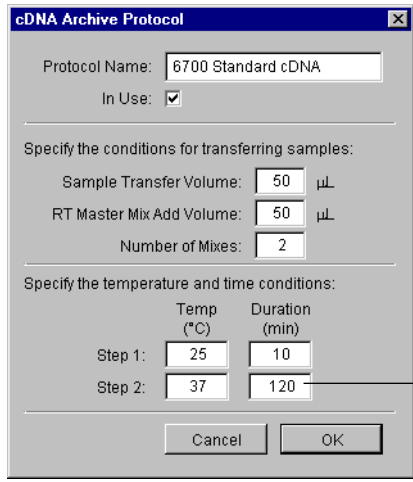
Step	Action
4	Place the 2X RT master mix on ice until you load the deckspace.

Setting Up the Instrument

Set up the 6700 workstation to prepare the cDNA archive plate.

Note See the *ABI PRISM 6700 Automated Nucleic Acid Workstation User's Manual* for help with setting up the instrument.

To set up the instrument:

Step	Action
1	Launch the 6700 system software, and log in.
2	Set up the **Protocol** tab. a. Select the cDNA archive protocol with the following conditions:  Recommended settings to achieve optimal conversion **IMPORTANT** For most applications and when large amounts of cDNA are required, Applied Biosystems recommends 120 minutes at 37 °C for reverse transcription to achieve optimal conversion. See "Yield vs. Reaction Time" on page 17 for more information. **Note** If your database does not contain a cDNA archive protocol with the conditions above, create the protocol. Use the *ABI PRISM 6700 Automated Nucleic Acid Workstation User's Manual* for instructions on how to create a cDNA archive protocol. b. Enter archive sample names by manual entry, by scanning the Consumable ID, or by importing sample names from a file.

To set up the instrument: *(continued)*

Step	Action
3	Begin cooling the deckspace by clicking the **Cool Peltiers** button on the **Instrument** tab.
4	Using the **Deckspace** tab of the system software and the barcode reader, load the deckspace with the following items: **IMPORTANT** Applied Biosystems recommends using only Applied Biosystems plastic consumables. Failure to do so may result in the robotic arm failing to sense liquid in the plate wells or in inaccurate pipetting. ♦ RNA archive plate containing at least 60 µL of RNA in each well designated to contain sample. **IMPORTANT** If any sample well contains less than 60 µL, the 6700 workstation will pause the run. ♦ 96-well reaction plate in the Dilution 1 position ♦ 2X RT master mix in the Master Mixes station ♦ Archive cover on the archive cover shelf ♦ Racks of disposable tips **Note** The amount of RT master mix and the number of racks of disposable tips required will vary, depending on how many RNA samples you are converting to cDNA.
5	Start the instrument run. a. Close the instrument door. b. Go to the **Instrument** tab of the software. c. Click the **Start** button. d. Make sure the instrument door is completely closed before proceeding. e. Click **OK**. f. Enter a name for the run. g. Click **OK**.

Manual Method

Procedure Overview

With the manual method, you perform the following:

- Prepare 2X RT master mix
- Manually prepare the cDNA archive reaction plate
- Place the plate in the GeneAmp® PCR System 9700 or 9600 thermal cycler for reverse transcription.

Preparing Reaction Master Mix

Prepare 2X RT master mix using the kit components before preparing the reaction plate.

To prepare 2X RT master mix:

Step	Action
1	Allow the kit components to thaw on ice.
2	Calculate the volume of components needed to prepare the cDNA archive reaction plate, using the table below. **Component** / **Volume (µL) / Reaction** 10X Reverse Transcription Buffer — 10 25X dNTPs — 4 10X random primers — 10 MultiScribe™ Reverse Transcriptase, 50 U/µL — 5 Nuclease-free H_2O — 21 **Total per Reaction** — 50 **Note** Include additional reactions in the calculations to provide excess volume for the loss that occurs during reagent transfers.
3	Pipette the required volumes of components into a 10-mL polypropylene tube. **⚠ WARNING CHEMICAL HAZARD. 10X RT Buffer** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate eyewear, clothing, and gloves.
4	Place the 2X RT master mix on ice until you prepare the cDNA archive reaction plate.

Preparing the cDNA Archive Reaction Plate

To prepare the cDNA archive reaction plate:

Step	Action
1	Pipette 50 µL of 2X RT master mix into each well of a 96-well reaction plate.
2	Pipette 50 µL of RNA sample into wells, pipetting up and down two times to mix.
3	Cover the plate with caps.
4	Briefly centrifuge the plate to spin down the contents and to eliminate any air bubbles.
5	Place the plate on ice until you are ready to load the thermal cycler.

Selecting a Thermal Cycler

Use one of the following instruments for PCR amplification:

- GeneAmp® PCR System 9700 thermal cycler
- GeneAmp® PCR System 9600 thermal cycler

IMPORTANT Because of the differences in ramp rates and thermal accuracy, you may need to adjust the settings if you choose to use other thermal cyclers.

Performing Reverse Transcription

To perform reverse transcription:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Program the thermal cycler conditions.

IMPORTANT These conditions are optimized for use with the High-Capacity cDNA Archive Kit.

<table><tr><th></th><th>Step 1</th><th>Step 2</th></tr><tr><td>Temperature</td><td>25 °C</td><td>37 °C</td></tr><tr><td>Time</td><td>10 min</td><td>120 min</td></tr></table>
IMPORTANT For most applications and when large amounts of cDNA are required, Applied Biosystems recommends 120 minutes at 37 °C for reverse transcription to achieve optimal conversion. See “Yield vs. Reaction Time” on page 17 for more information.</td></tr>
<tr><td>2</td><td>Set the reaction volume to 100 µL.</td></tr>
<tr><td>3</td><td>Load the reaction plate into the thermal cycler.</td></tr>
<tr><td>4</td><td>Start the reverse transcription run.</td></tr>
</table>

cDNA Archive Plate Usage Recommendations

Output Applications

The cDNA archive plates prepared using the High-Capacity cDNA Archive Kit can be used in a variety of applications, including

- Short-term and long-term archival storage
- Quantitative PCR
- Conversion to cRNA

Storing cDNA Archive Plates

You can store cDNA archive plates prepared using the High-Capacity cDNA Archive Kit for short-term or long-term storage.

Term	Temperature
Short-term (up to 24 hours before use)[a]	2 to 6 °C
Long-term	–15 to –25 °C

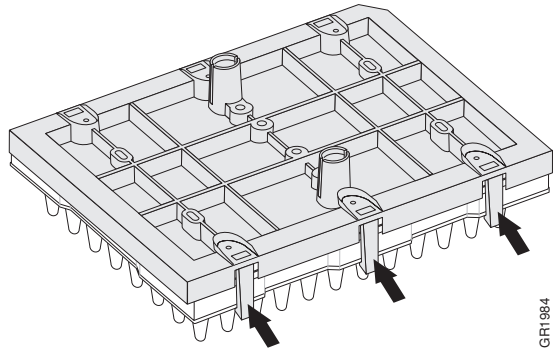
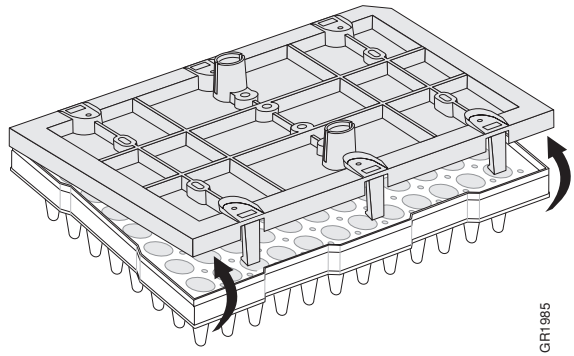
a. For prolonged storage at 2 to 6 °C, add EDTA to a final concentration of 1mM to chelate cations and to prevent nucleic acid degradation.

Note If required, briefly centrifuge the archive plates before storing to spin down the contents and to eliminate any air bubbles.

Removing cDNA Archive Plates from Storage

If the cDNA archive plate is covered with an archive cover, follow the procedure below to remove the archive cover.

To remove the archive cover:

Step	Action
1	Unclip the front three clips. 
2	Carefully tilt back the cover, and lift it off. 

Appendix A. Examples of cDNA Conversion Performance

Methods for Analysis

To evaluate the yield of the conversion of total RNA to cDNA, use one of the following methods:

- Quantitative PCR
- PicoGreen® Quantitation Reagent (Molecular Probes, Eugene, Oregon)[1]

Quantitative PCR

To determine the yield of the cDNA conversion, use quantitative PCR methods to test various input amounts of RNA for cDNA yield of different gene targets. The table below lists some targets and Applied Biosystems kits that you can use to evaluate the yield of cDNA conversion.

Gene Target	Kit	P/N
18S	TaqMan® Ribosomal RNA Control Reagents	4308329
GAPDH	TaqMan® GAPDH Control Reagents [Human]	402869
GAPDH	TaqMan® Rodent GAPDH Control Reagents	4308313
β-actin	TaqMan® β-actin Detection Reagents	401846

You can use other Pre-Developed TaqMan® Assay Reagents for Gene Expression to evaluate the yield of cDNA conversion. Visit our Web site (www.appliedbiosystems.com/pdarlist) for a list of available assays.

1. Seville, M., West, A.B., Cull, M.G., and McHenry, C.S. 1996. Fluorometric assay for DNA polymerases and reverse transcriptase. *Biotechniques* 21:664–672.

Yields for Different Targets

For the example in this section, the input total RNA was obtained from human Raji cells, and the RNA was converted to cDNA using the High-Capacity cDNA Archive Kit.

Figure 1 shows an example of results of quantitative PCR of the cDNA for 11 different gene targets, which vary in expression levels.

Note The amplicon for β_2-microglobulin in this study was specifically designed to be extremely A/U rich.

The threshold cycle (C_T) values are plotted against RNA input of 0.1, 1.0, and 10.0 µg.

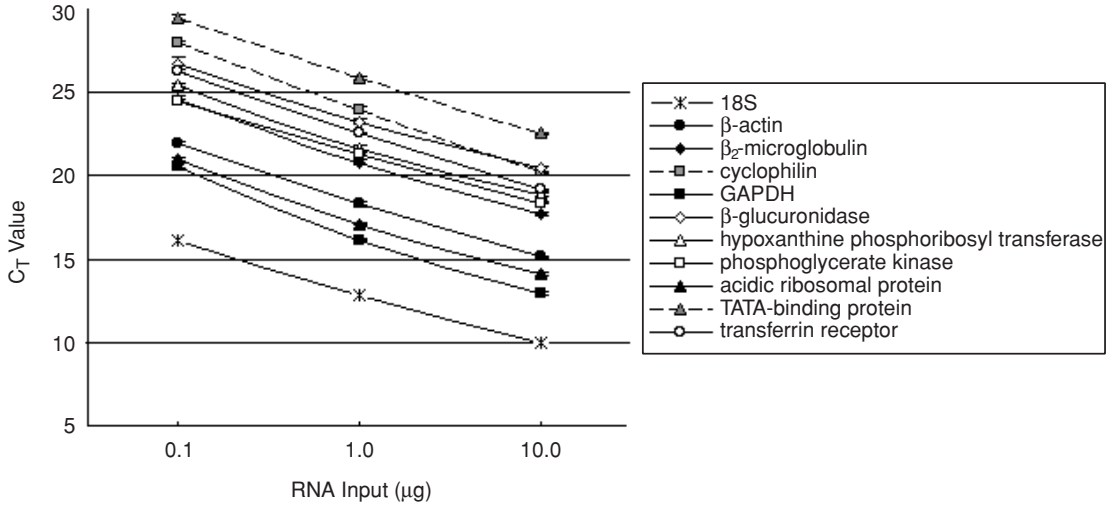


Figure 1 The expected ΔC_T value of 3.3 for each tenfold increase in input quantity is obtained for 11 different RNA transcripts converted to cDNA from different input quantities of total RNA.

Yield vs. Reaction Time

To achieve optimal conversion, Applied Biosystems recommends allowing reverse transcription to occur for 120 minutes at 37 °C.

Figures 2, 3, and 4 show C_T values plotted against reaction time (minutes) for three different targets (18S, GAPDH, and β_2-microglobulin) and three input amounts of RNA (0.1, 1.0, and 10.0 µg).

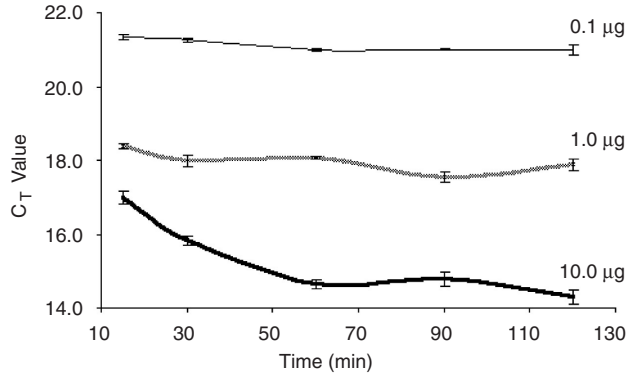


Figure 2 The conversion of 18S RNA to cDNA reaches a maximum at 120 minutes with 10 µg of input RNA and at 30 minutes or less with 0.1–1.0 µg of input RNA.

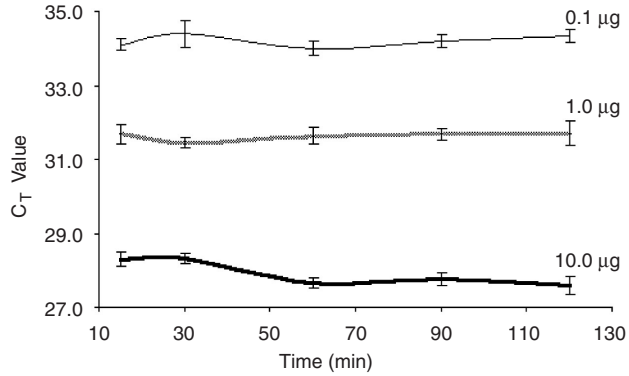


Figure 3 The conversion of GAPDH RNA to cDNA reaches a maximum at 60 minutes or less at all RNA input levels.

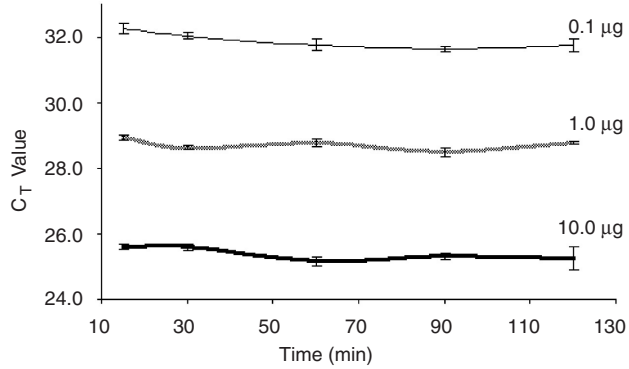


Figure 4 The conversion of β_2-microglobulin RNA to cDNA reaches a maximum at 60 minutes or less at all RNA input levels.

Appendix B. Technical Support

Contacting Technical Support

You can contact Applied Biosystems for technical support by telephone or fax, by e-mail, or through the Internet. You can order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents 24 hours a day. In addition, you can download documents in PDF format from the Applied Biosystems Web site (please see the section "To Obtain Documents on Demand" following the telephone information below).

To Contact Technical Support by E-Mail

Contact technical support by e-mail for help in the following product areas:

Product Area	E-mail address
Genetic Analysis (DNA Sequencing)	galab@appliedbiosystems.com
Sequence Detection Systems and PCR	pcrlab@appliedbiosystems.com
Protein Sequencing, Peptide and DNA Synthesis	corelab@appliedbiosystems.com
Biochromatography, PerSeptive DNA, PNA and Peptide Synthesis systems, CytoFluor®, FMAT™, Voyager™, and Mariner™ Mass Spectrometers	tsupport@appliedbiosystems.com
Applied Biosystems/MDS Sciex	api3-support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

Hours for Telephone Technical Support

In the United States and Canada, technical support is available at the following times:

Product	Hours
Chemiluminescence	8:30 a.m. to 5:30 p.m. Eastern Time
Framingham support	8:00 a.m. to 6:00 p.m. Eastern Time
All Other Products	5:30 a.m. to 5:00 p.m. Pacific Time

To Contact Technical Support by Telephone or Fax

In North America

To contact Applied Biosystems Technical Support, use the telephone or fax numbers given below. (To open a service call for other support needs, or in case of an emergency, dial **1-800-831-6844** and press **1**.)

Product or Product Area	Telephone Dial...	Fax Dial...
ABI PRISM® 3700 DNA Analyzer	**1-800-831-6844,** then press **8**	**1-650-638-5981**
DNA Synthesis	**1-800-831-6844**, then press **21**	**1-650-638-5981**
Fluorescent DNA Sequencing	**1-800-831-6844**, then press **22**	**1-650-638-5981**
Fluorescent Fragment Analysis (includes GeneScan® applications)	**1-800-831-6844**, then press **23**	**1-650-638-5981**
Integrated Thermal Cyclers (ABI PRISM®877 and Catalyst 800 instruments)	**1-800-831-6844**, then press **24**	**1-650-638-5981**
ABI PRISM® 3100 Genetic Analyzer	**1-800-831-6844**, then press **26**	**1-650-638-5981**
BioInformatics (includes BioLIMS™, BioMerge™, and SQL GT™ applications)	**1-800-831-6844**, then press **25**	**1-505-982-7690**
Peptide Synthesis (433 and 43X Systems)	**1-800-831-6844**, then press **31**	**1-650-638-5981**
Protein Sequencing (Procise® Protein Sequencing Systems)	**1-800-831-6844**, then press **32**	**1-650-638-5981**
PCR and Sequence Detection	**1-800-762-4001**, then press **1** for PCR, **2** for the 7700 or 5700, **6** for the 6700 or dial **1-800-831-6844**, then press **5**	**1-240-453-4613**

Product or Product Area	Telephone Dial...	Fax Dial...
Voyager™ MALDI-TOF Biospectrometry and Mariner™ ESI-TOF Mass Spectrometry Workstations	**1-800-899-5858**, then press **13**	**1-508-383-7855**
Biochromatography (BioCAD® Workstations and Poros® Perfusion Chromatography Products)	**1-800-899-5858**, then press **14**	**1-508-383-7855**
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Hong Kong	**852 2756 6928**	**852 2756 6968**
Korea (Seoul)	**82 2 593 6470/6471**	**82 2 593 6472**
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Singapore	**65 896 2168**	**65 896 2147**
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Thailand (Bangkok)	**66 2 719 6405**	**66 2 319 9788**
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Russia (Moskva)	**7 095 935 8888**	**7 095 564 8787**
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Japan (Hacchobori, Chuo-Ku, Tokyo)	**81 3 5566 6006**	**81 3 5566 6505**
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