Applied Biosystems Chemiluminescent RT Labeling Kit

Protocol

Applied Biosystems

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

TRADEMARKS:

Part Number 4339628 Rev. C
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Contents

Preface

This preface contains:

Safety

Safety Alert Words

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

IMPORTANT! – Indicates information that is necessary for proper instrument operation, 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.

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.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page vi.)
- 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 MSDS.
- 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 MSDS.
- 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.

About MSDSs

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

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

Obtaining MSDSs

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS 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
4. To have a copy of a document sent by fax or e-mail, select **Fax** or **Email** to the left of the document title in the Search Results page, then click **RETRIEVE DOCUMENTS** at the end of the document list.
5. After you enter the required information, click **View/Deliver Selected Documents Now**.

Chemical Waste Hazard

CHEMICAL WASTE HAZARD. Some wastes produced by the operation of the instrument or system are potentially hazardous and can cause injury, illness, or death.

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) 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 MSDS.
- 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 MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the 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

BIOHAZARD. Biological samples such as tissues, body fluids, 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 eyewear, clothing, and gloves. Read and follow the guidelines in these publications:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **http://bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**).

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

How to Obtain Support

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Overview

Purpose

The purpose of the Applied Biosystems Chemiluminescent RT Labeling Kit (PN 4340415) is to convert poly(A) mRNA into digoxigenin (DIG)-labeled cDNA for hybridization to Applied Biosystems microarrays.

Kit Contents

The RT labeling kit contains:

- RT labeling reagents
- cDNA purification reagents, columns, and tubes

About This Protocol

This protocol provides the following:

- Information about kit capacity, components, quantity, storage, and stability
- A list of required and optional equipment and materials not supplied with the kit
- Procedures for using the RT labeling kit
- Troubleshooting information

Process Illustration

Figure 1 illustrates the RT labeling process using the RT labeling kit.

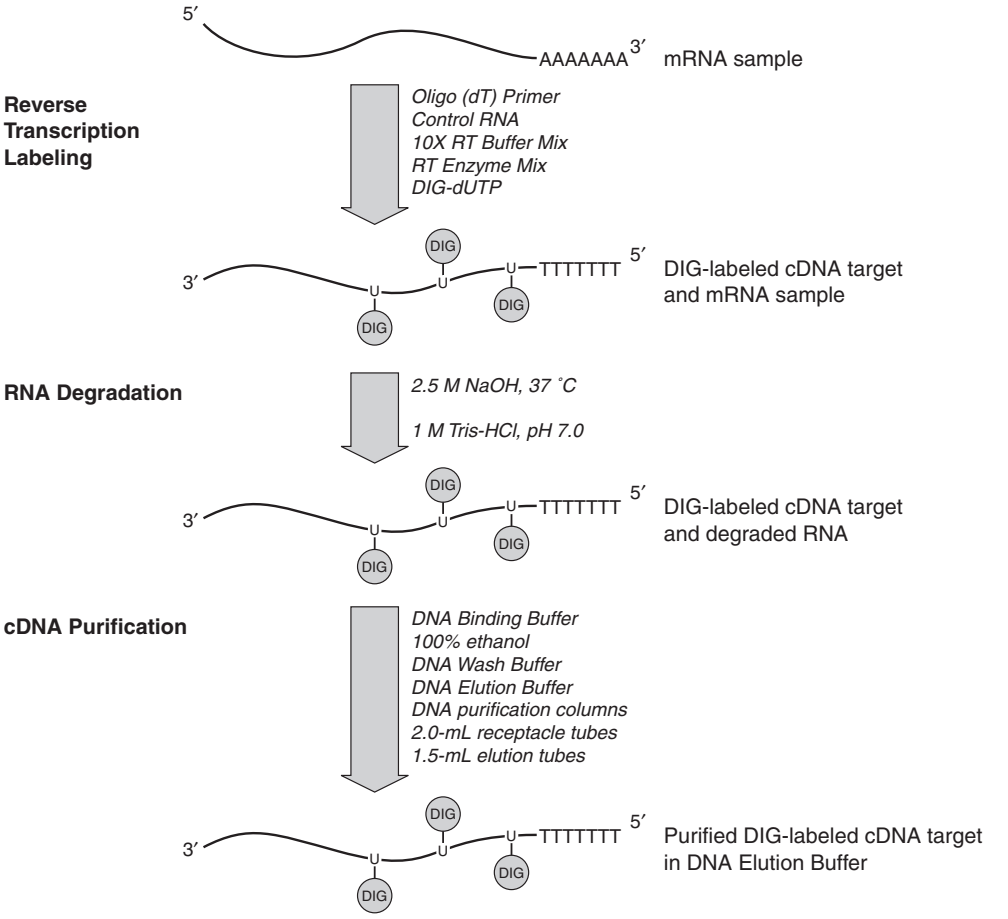

Figure 1 RT labeling process

Materials and Equipment

Kit Capacity

The Applied Biosystems Chemiluminescent RT Labeling Kit (PN 4340415) contains materials sufficient to prepare and purify DIG-labeled cDNA from 12 RNA samples.

Components

The kit is packaged in two boxes:

- Applied Biosystems Chemiluminescent RT Labeling Reagents
- Applied Biosystems Chemiluminescent RT Purification Components

RT Labeling Reagents

Use the RT labeling reagents for "Performing Reverse Transcription Labeling" on page 8.

Store the RT labeling kit components at –15 °C to –25 °C.

Table 1 RT labeling reagents

Reagent	Contents	Quantity
10X RT Buffer Mix	• dATP, dCTP, and dGTP (5.0 mM each) • dTTP (4.33 mM)	36 µL
Nuclease-free water	Nuclease-free water	2 mL
Oligo (dT) Primer	Oligo (dT) primers (50.0 µM)	36 µL
RT Enzyme Mix	• Reverse transcriptase enzyme (33.3 U/µL) • RNase inhibitor (6.7 U/µL)	36 µL
Control RNA	Bacterial mRNA transcripts with poly(A) tails: • *lys* — 1100 nt (0.5 nM) • *phe* — 1400 nt (0.5 nM) • *dap* — 1900 nt (0.5 nM)	24 µL

RT Purification Components

Use RT purification components for:

- "Degrading RNA" on page 10
- "Purifying cDNA" on page 11

Store the RT purification components at ambient temperature.

Table 2 RT purification components

Component	Quantity
1.5-mL elution tubes	12 tubes
1M Tris-HCl, pH 7.0	0.5 mL
2.0-mL receptacle tubes	12 tubes
2.5 M NaOH	60 µL
DNA Binding Buffer	1.2 mL
DNA Elution Buffer	4 mL
DNA purification columns	12 columns
DNA Wash Buffer	18 mL

Equipment and Materials Not Included

Table 3 and Table 4 include required and optional equipment and materials not supplied with the RT labeling kit. Unless otherwise noted, many of the items are available from major laboratory suppliers (MLS).

Table 3 Applied Biosystems instruments

Instrument	Source
Applied Biosystems 1700 Chemiluminescent Microarray Analyzer	Contact your local Applied Biosystems sales representative.
96-Well GeneAmp® PCR System 9700	

Table 4 User-supplied materials

Material	Source
MicroAmp® Reaction Tubes With Caps (1,000 tubes)	Applied Biosystems (PN N801-0540)
MicroAmp® Autoclaved Reaction Tubes With Caps (1,000 tubes)	Applied Biosystems (PN N801-0612)
MicroAmp® Colored Reaction Tubes With Caps (1,000 tubes)	Applied Biosystems (PN N801-0840)
Bulkpack MicroAmp® Reaction Tubes With Caps (10,000 tubes)	Applied Biosystems (PN N801-1540)
Digoxigenin-11-deoxyuridine-5′-triphosphate solution (DIG-dUTP): 25 nmol (25 µL)	Roche Molecular Biochemicals (Cat. No. 1 093 088)
Ethanol, 100%	MLS
Microcentrifuge	MLS
Microcentrifuge tubes, nuclease-free: 1.5-mL	MLS
Pipette tips, nuclease-free: 1- to 20-µL range, 20- to 200-µL range, 100- to 1000-µL range	MLS
Pipettors: 1- to 20-µL range, 20- to 200-µL range, 100- to 1000-µL range	MLS
Vortexer	MLS

Applied Biosystems Documents

Table 5 Applied Biosystems documents

Document	Part Number
Applied Biosystems 1700 Chemiluminescent Microarray Analyzer Chemistry Guide	4338853
Applied Biosystems 1700 Chemiluminescent Microarray Analyzer Site Preparation Guide	4338850
Applied Biosystems 1700 Chemiluminescent Microarray Analyzer User Guide	4338852
Applied Biosystems Chemiluminescence Detection Kit Protocol	4339627
Applied Biosystems Chemiluminescence Detection Kit Quick Reference Card	4346875
Applied Biosystems Chemiluminescent RT Labeling Kit Quick Reference Card	4346876
Applied Biosystems Chemiluminescent RT-IVT Labeling Kit Protocol	4339629
Applied Biosystems Chemiluminescent RT-IVT Labeling Kit Quick Reference Card	4346877

RT Labeling Procedures

Procedure Overview

Preparing and purifying DIG-labeled cDNA from mRNA samples using the RT labeling kit involves:

1. Performing reverse transcription labeling (page 8)
2. Degrading RNA (page 10)
3. Purifying cDNA (page 11)

Procedure Guidelines

For optimal performance of the RT labeling kit:

- Use RNA sample with the following characteristics:
  - Quantity: 2 to 5 µg of mRNA sample
  - Amount of genomic DNA by weight: Less than 0.005%
  - Free of reverse transcription inhibitors
  - Free of RNase activity
- Use nuclease-free pipette tips and reagents to minimize degradation of the RNA sample and the cDNA product.
- Observe standard laboratory practices when handling RNA.

Selecting a Thermal Cycler

For each step that requires a thermal cycler, use the GeneAmp PCR System 9700. The heated lid minimizes evaporation and condensation in the tube cap.

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 Labeling

Use the RT labeling reagents to perform reverse transcription labeling.

To perform reverse transcription labeling:

1. Prepare sample and labeling reagents:
   a. Thaw on ice:
      - mRNA sample (2 to 5 µg)
      - Oligo (dT) Primer
      - Control RNA
      - Nuclease-free water
   b. Vortex tubes, then centrifuge briefly.

2. Pipette the components into a 0.2-mL MicroAmp reaction tube on ice:

Component	Volume (µL)
Oligo (dT) Primer	3.0
Control RNA	2.0
mRNA sample (2 to 5 µg) and nuclease-free water	17.0
Total	22.0

3. Heat and cool the RNA and primer mixture in a thermal cycler:
   a. Program the thermal cycler conditions:

Stage	Description	Temp.	Time
1	Melting	70 °C	5 minutes
2	Primer annealing	4 °C	Indefinite hold

   b. Set the reaction volume to **22 µL**.
   c. Load the tube into the thermal cycler and start the run.
   d. After the run, place the tube on ice.

To perform reverse transcription labeling: ***(continued)***

4. Check the 10X RT Buffer Mix for precipitates.

   Note: If precipitates are present, warm the buffer at 37 °C for 2 to 3 minutes, then vortex it briefly before using.

   WARNING CHEMICAL HAZARD. 10X RT Buffer Mix contains dithiothreitol. Exposure may cause nervous system depression. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

5. Add the following components to the reaction tube and mix thoroughly by pipetting:

Component	Volume (µL)
10X RT Buffer Mix	3.0
DIG-dUTP (approximately 2 nmol)	2.0
RT Enzyme Mix	3.0

6. Perform reverse transcription in the thermal cycler:

   a. Program the thermal cycler conditions.

      IMPORTANT! These conditions are optimized for use with the RT labeling kit.

Stage	Description	Temp.	Time
1	Initiation	25 °C	10 minutes
2	Extension	42 °C	3 hours
3	Enzyme inactivation	70 °C	15 minutes
4	Hold	4 °C	Indefinite hold

   b. Set the reaction volume to **30 µL**.

   c. Load the tube into the thermal cycler and start the run.

To perform reverse transcription labeling: *(continued)*

7.	After the run, remove the tube from the thermal cycler.

Degrading RNA

User reagents from the cDNA purification components to degrade the RNA.

To degrade RNA:

1.	Add 3.0 µL of 2.5 M NaOH to the reaction tube and mix thoroughly by pipetting.  **DANGER CHEMICAL HAZARD. Sodium hydroxide (NaOH)** causes severe eye, skin, and respiratory tract burns. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2.	Perform RNA degradation in the thermal cycler: a. Program the thermal cycler conditions: • Temperature: 37 °C • Time: 15 minutes • Reaction volume: 33 µL b. Load the tube into the thermal cycler and start the run.
3.	*Immediately* after the 15-minute incubation, add 20 µL of 1 M Tris-HCl, pH 7.0 to the reaction tube and mix thoroughly to neutralize the reaction.

Purifying cDNA

Use cDNA purification components to purify the cDNA.

IMPORTANT! Handle the DNA purification column by the top edges to prevent contamination. Do not touch the fiber matrix.

To purify the cDNA:

1.	In a new 1.5-mL nuclease-free microcentrifuge tube, combine: • DNA Binding Buffer: 100 µL • 100% ethanol: 100 µL **DANGER** **CHEMICAL HAZARD**. **DNA Binding Buffer** contains guanidine thiocyanate. Exposure causes burns and skin irritation. It is harmful if absorbed through the skin or if swallowed. Contact with acids or bleach liberates toxic gases. DO NOT ADD acids or bleach to any liquid wastes containing DNA Binding Buffer. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **WARNING** **CHEMICAL HAZARD**. **Ethanol** is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation and may cause central nervous system depression and liver damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2.	Add the entire RT reaction (53 µL) to the DNA Binding Buffer-ethanol mixture and mix thoroughly by pipetting.

To purify the cDNA: ***(continued)***

3.	Begin the purification: a. Insert a DNA purification column into a 2-mL receptacle tube. b. Transfer the RT reaction-DNA Binding Buffer-ethanol mixture (253 µL) to the column, then close the tube. c. Centrifuge the column and tube at 13,000 × *g* for 1 minute. d. Make sure that the entire volume passed through the column. If it did not, centrifuge the column and tube at 13,000 × *g* for 1 minute. e. Remove the column from the tube, discard the liquid, then reinsert the column into the tube.
4.	Wash the cDNA (1): a. Add 700 µL of DNA Wash Buffer to the column, then close the tube. b. Centrifuge the column and tube at 13,000 × *g* for 1 minute. c. Remove the column from the tube, discard the liquid, then reinsert the column into the tube.  **WARNING CHEMICAL HAZARD. DNA Wash Buffer** contains ethanol. It is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation, and may cause central nervous system depression and liver damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To purify the cDNA: ***(continued)***

5.	Wash the cDNA (2): a. Add 700 µL of DNA Wash Buffer to the column, then close the tube. b. Centrifuge the column and tube at 13,000 × *g* for 1 minute. c. Remove the column from the tube, discard the liquid, then reinsert the column into the tube. d. Centrifuge the column and tube at 13,000 × *g* for 1 minute.  **WARNING CHEMICAL HAZARD. DNA Wash Buffer** contains ethanol. It is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation, and may cause central nervous system depression and liver damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
6.	Elute the cDNA (1): a. Transfer the column to a new 1.5-mL elution tube. b. Pipette 30 µL of DNA Elution Buffer onto the fiber matrix at the bottom of the column, then close the tube. **IMPORTANT!** Do not let the pipette tip touch the fiber matrix. c. Incubate the column at room temperature for 1 minute. d. Centrifuge the column and tube at 13,000 × *g* for 1 minute for an elution volume of 30 µL.

To purify the cDNA: ***(continued)***

7.	Elute the cDNA (2): a. Pipette 30 µL of DNA Elution Buffer onto the fiber matrix at the bottom of the column, then close the tube. **IMPORTANT!** Do not let the pipette tip touch the fiber matrix. b. Incubate the column at room temperature for 1 minute. c. Centrifuge the column and tube at 13,000 × *g* for 1 minute for an elution volume of 60 µL.
8.	Elute the cDNA (3): a. Pipette 30 µL of DNA Elution Buffer onto the fiber matrix at the bottom of the column, then close the tube. **IMPORTANT!** Do not let the pipette tip touch the fiber matrix. b. Incubate the column at room temperature for 1 minute. c. Centrifuge the column and tube at 13,000 × *g* for 1 minute for a final elution volume of 90 µL. d. Discard the column, then close the tube.

Storing cDNA Product

Store the cDNA product at:

- –15 to –25 °C for up to 2 months or
- –80 °C for long-term storage

Troubleshooting

If the level of chemiluminescent signals from the gene expression probes is low, check the level of chemiluminescent signals from the RT control probes (*lys, phe,* and *dap*). Use Table 6 to identify possible causes of the problem and to determine recommended actions to resolve the problem.

Table 6 Troubleshooting

Level of Chemiluminescent Signals	Possible Cause	Action
Gene expression probes: low RT control probes: good	RNA sample does not meet the guidelines on page 7.	Extract more RNA from your samples and make sure that it meets the guidelines before proceeding.
Gene expression probes: low RT control probes: low	RNase activity in RT labeling reagents or chemiluminescence detection reagents.	1. Test reagents for RNase activity. 2. Discard all contaminated reagents. 3. Repeat the experiment using RNase-free products.
	Insufficient incorporation of DIG-dUTP.	Measure DIG incorporation.
	10X RT Buffer Mix contained precipitates.	Check the buffer mix for precipitates. If precipitates are present, make sure that you warm the buffer mix and vortex it well before using. See page 9 for the step that describes how to dissolve precipitates.
	Inactive enzyme failed to convert RNA to cDNA.	Make sure that you store the enzyme properly. See Table 1 on page 3 for storage conditions of kit components.
	DIG-UTP was used instead of DIG-dUTP.	Use the correct DIG product. See page 5 for the product description and ordering information.

Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

Worldwide Sales and Support
Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches 150 countries on six continents. For sales office locations and technical support, please call our local office or refer to our Web site at www.appliedbiosystems.com.

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