USER GUIDE

invitrogen™
by life technologies™

SuperScript™ Direct cDNA Labeling System

For generating fluorescently labeled cDNA to use in microarray screening

Catalog nos. L1015-01, L1015-02, and L1015-03

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

ThermoFisher SCIENTIFIC

Table of Contents

Kit Contents and Storage

Kit Sizes

The SuperScript™ Direct cDNA Labeling System is supplied with either a Core Module and a Purification Module, or a Core Module only. Note that the Core Module contains the labeling components.

Cat no.	Number of Labeling Reactions	Modules
L1015-01	10	Core and Purification
L1015-02	30	Core and Purification
L1015-03	30	Core only

Shipping and Storage

The Core Module is shipped on dry ice and the Purification Module is shipped at room temperature. Upon receipt, store the components of the Core Module at -20°C and store the components of the Purification Module at room temperature.

Core Module

The components of the Core Module should be stored at -20°C.

Item	Components/Concentration	Kit Size	
		10 Rxn	30 Rxns
SuperScript™ III Reverse Transcriptase	400 U/µl in: 20 mM Tris-HCl (pH 7.5) 100 mM NaCl 0.1 mM EDTA 1 mM DTT 0.01% (v/v) NP-40 50% (v/v) glycerol	25 µl	70 µl
5X First-Strand Buffer	250 mM Tris-HCl (pH 8.3, room temp) 375 mM KCl 15 mM $MgCl_2$	1000 µl	1000 µl
Dithiothreitol (DTT)	0.1 M DTT in water	500 µl	500 µl
dNTP Mix for Labeled dCTP	dATP, dGTP, dCTP, dTTP at optimal concentrations in DEPC-treated water	25 µl	70 µl
dNTP Mix for Labeled dUTP	dATP, dGTP, dCTP, dTTP at optimal concentrations in DEPC-treated water	25 µl	70 µl
Anchored Oligo$(dT)_{20}$ primer	2.5 µg/µl in DEPC-treated water	25 µl	70 µl
Random hexamer primers	0.5 µg/µl in DEPC-treated water	11 µl	40 µl
RNaseOUT™	40 U/µl	11 µl	70 µl
DEPC-treated Water	—	2 ml	6 ml
Control HeLa RNA	1 µg/µl in HE buffer	10 µg	10 µg

Continued on next page

Kit Contents and Storage, continued

Purification Module

The components of the Purification Module should be stored at room temperature. Note that the 30-reaction kit contains three boxes of the Purification Module supplied in the 10-reaction kit. This module is included with Catalog Numbers L1015-01 and L1015-02.

Item	Kit Size	
	10 Rxns	30 Rxns
Loading Buffer (you must add 100% isopropanol to create the final buffer; see below)	4.2 ml	3 × 4.2 ml
Wash Buffer (you must add 100% ethanol to create the final buffer; see below)	2 ml	3 × 2 ml
Purification Columns	11 cols	3 × 11 cols
Amber Collection Tubes	11 tubes	3 × 11 tubes

Preparing Loading Buffer with Isopropanol

The Loading Buffer supplied in each Purification Module must be mixed with 100% isopropanol prior to use. The Loading Buffer plus isopropanol is stable for at least six months at room temperature.

Add the amount of isopropanol indicated below directly to each bottle of Loading Buffer. Be sure to mark the appropriate checkbox on the bottle to indicate that you have added the isopropanol.

Component	Amount
Loading Buffer	4.2 ml (entire bottle)
100% Isopropanol	1.4 ml
Final Volume	5.6 ml

Note: For the 30-reaction kit, we recommend preparing all three Loading Buffer bottles with isopropanol at the same time to avoid interruptions in the purification procedure.

Preparing Wash Buffer with Ethanol

The Wash Buffer supplied in each Purification Module must be mixed with 100% ethanol prior to use. The Wash Buffer plus ethanol is stable for at least six months at room temperature.

Add the amount of ethanol indicated below directly to each bottle of Wash Buffer. Be sure to mark the appropriate checkbox on the bottle to indicate that you have added the ethanol.

	Amount
Wash Buffer	2 ml (entire bottle)
100% Ethanol	6 ml
Final Volume	8 ml

Note: For the 30-reaction kit, we recommend preparing all three Wash Buffer bottles with ethanol at the same time to avoid interruptions in the purification procedure.

Product Qualification

This kit was verified using 10 µg total HeLa RNA in a standard labeling reaction with Cy3™- or Cy5™-labeled dCTP. After purification, the labeled cDNA was scanned to read the full absorbance spectrum from 240–800 nm, using dH_2O as a blank. The amounts of incorporated nucleotides were calculated using the formulas on page 8. In addition, the length of the labeled product was determined by gel electrophoresis.

Overview

Introduction

The SuperScript™ Direct cDNA Labeling System is a highly robust and efficient system for generating fluorescently labeled cDNA for use on microarrays in gene expression studies. It uses SuperScript™ III Reverse Transcriptase in a cDNA synthesis reaction with total RNA or mRNA, an optimized dNTP mixture, and a fluorescently labeled nucleotide of your choice (labeled dCTP or dUTP). After cDNA synthesis, the RNA template is hydrolyzed, a purification step removes any unincorporated nucleotides, and the fluorescently labeled cDNA is ready for hybridization to microarrays.

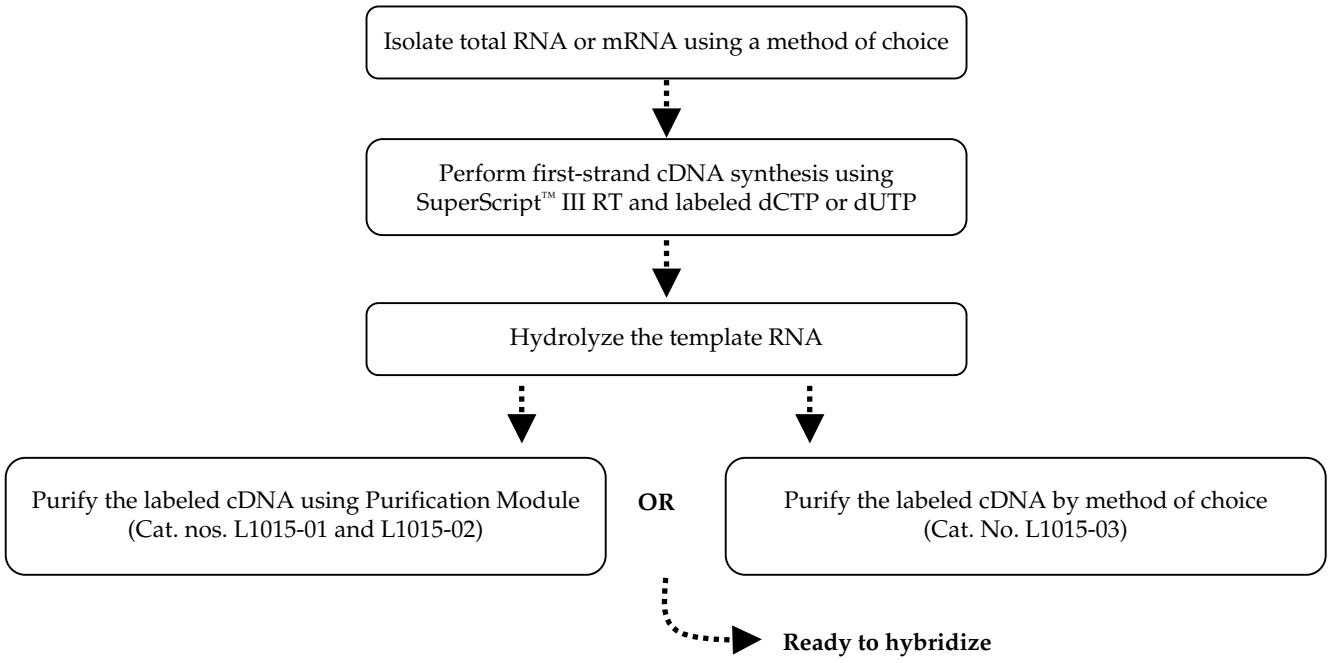
This system has been optimized for use with 10–40 µg of total RNA or 0.4–2 µg of mRNA as starting material. Lower amounts of starting material may be used, but may result in lower hybridization signals. This kit is compatible with Cy3™- and Cy5™-labeled nucleotides from Amersham Biosciences or fluorescently labeled nucleotides from other manufacturers.

Advantages of the System

- Optimized reagents and protocol ensure highly robust and reproducible labeling reactions
- SuperScript™ III Reverse Transcriptase in the first-strand synthesis reaction produces high yields of cDNA, greater incorporation of fluorescent nucleotides, and higher signal-to-background ratios with small amounts of starting material
- An optimal ratio of labeled dNTP to unlabeled dNTP results in an even distribution of fluorescent signal and high overall levels of fluorescence, increasing the sensitivity and reproducibility of hybridizations
- System includes all major reagents and materials for preparing fluorescently labeled cDNA, except fluorescent nucleotides

Experimental Outline

The flow chart below outlines the experimental steps of the system:



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Overview, continued

Advantages of SuperScript™ III Reverse Transcriptase

SuperScript™ III Reverse Transcriptase is an engineered version of M-MLV RT with reduced RNase H activity and increased thermal stability. The enzyme can be used to synthesize first-strand cDNA from total RNA or mRNA at temperatures up to 55°C, providing increased specificity, higher yields of cDNA, and more full-length product than other reverse transcriptases.

The SuperScript™ III RT in this kit is provided at an optimal concentration and used at an optimal temperature for incorporating labeled nucleotides in first-strand cDNA synthesis.

Anchored Oligo(dT)$_{20}$

Anchored oligo(dT)$_{20}$ primer is a mixture of 12 primers, each consisting of a string of 20 deoxythymidylic acid (dT) residues followed by two additional nucleotides represented by VN, where:

- V is dA, dC, or dG
- N is dA, dC, dG or dT

The VN "anchor" allows the primer to anneal only at the 5′ end of the poly(A) tail of mRNA, providing more efficient cDNA synthesis for labeling applications.

Labeled Nucleotides

This system is compatible with labeled dCTP or dUTP from a variety of manufacturers. It has been developed using the following CyDye™ fluorescent nucleotides:

Alexa Fluor® 647-aha-dUTP, 1 mM (Cat. no. A32763)
Alexa Fluor® 555-aha-dUTP, 1 mM (Cat. no. A32762)

Cy3™-dCTP, 1 mM (Amersham Biosciences, #PA53021)
Cy3™-dUTP, 1 mM (Amersham Biosciences, #PA53022)
Cy5™-dCTP, 1 mM (Amersham Biosciences, #PA55021)
Cy5™-dUTP, 1 mM (Amersham Biosciences, #PA55022)

Materials Supplied by the User

In addition to the kit components, you should have the following items on hand before using the SuperScript™ Direct cDNA Labeling System.

- 10–40 µg total RNA or 0.4–2 µg mRNA starting material
- Fluorescently labeled nucleotides
- Vortex mixer
- Microcentrifuge
- Aerosol resistant pipette tips
- Water baths or incubator
- 0.1 M NaOH
- 0.1 M HCl
- 0.5 or 1.5-ml RNase-free microcentrifuge tubes
- 100% Isopropanol
- 100% Ethanol

Control RNA

Control HeLa RNA is included in the kit to help you determine the efficiency of the labeling procedure. We recommend that you perform the complete labeling procedure using the control HeLa RNA if you are a first-time user of the system.

Equations for calculating the efficiency of the labeling procedure are provided on page 8.

Methods

Isolating RNA

Introduction

High-quality, intact RNA is essential for full-length, high-quality cDNA synthesis. In this step, you isolate total RNA or mRNA using a method of choice.

Important

The quality of the RNA is critical for successful labeling and hybridization. The presence of contaminants in the RNA may significantly increase background fluorescence in your microarrays. Carefully follow the recommendations below to prevent RNase contamination.

General Handling of RNA

When working with RNA:

- Use disposable, individually wrapped, sterile plasticware.
- Use aerosol resistant pipette tips for all procedures.
- Use only sterile, new pipette tips and microcentrifuge tubes.
- Wear latex gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin.
- Use proper microbiological aseptic technique when working with RNA.
- Dedicate a separate set of pipettes, buffers, and enzymes for RNA work.
- Microcentrifuge tubes can be taken from an unopened box, autoclaved, and used for all RNA work. RNase-free microcentrifuge tubes are available from several suppliers. If it is necessary to decontaminate untreated tubes, soak the tubes overnight in a 0.01% (v/v) aqueous solution of diethylpyrocarbonate (DEPC), rinse the tubes with sterile distilled water, and autoclave the tubes.

You can use RNase *AWAY*™ Reagent, a non-toxic solution available from Invitrogen (see page 11), to remove RNase contamination from surfaces. For further information on controlling RNase contamination, see Ausubel, *et al.*, 1994, and Sambrook, *et al.*, 1989.

Isolating RNA

This system is optimized for use with 10–40 µg total RNA or 0.4–2 µg of mRNA. Lower amounts of starting material may be used, but may result in lower hybridization signals. To isolate total RNA, we recommend TRIzol® Reagent (Chirgwin *et al.*, 1979; Chomczynski and Sacchi, 1987) or the Micro-to-Midi Total RNA Purification System. To isolate mRNA, we recommend the Micro-FastTrack™ 2.0 or FastTrack® 2.0 mRNA Isolation Kits. Ordering information is provided on page 11.

After you have isolated the RNA, check the quality of your RNA preparation as described on the following page.

Continued on next page

Isolating RNA, continued

Checking the RNA Quality

To check RNA quality, analyze 500 ng of RNA by agarose/ethidium bromide gel electrophoresis. You can use a regular 1% agarose gel or a denaturing agarose gel (Ausubel *et al.*, 1994). For total human RNA using a regular agarose gel, mRNA will appear as a smear from 0.5 to 9 kb, and 28S and 18S rRNA will appear as bands at 4.5 kb and 1.9 kb, respectively. The 28S band should be twice the intensity of the 18S band. If you are using a denaturing gel, the rRNA bands should be very clear and sharp.

If you do not load enough RNA, the 28S band may appear to be diffuse. A smear of RNA or a lower intensity 28S band with an accumulation of low molecular weight RNA on the gel are indications that the RNA may be degraded, which will decrease the labeling efficiency. If you do not detect any RNA, you will need to repeat RNA isolation. Refer to the **Troubleshooting** section on page 10.

Storing RNA

After preparing the RNA, we recommend that you proceed directly to **First-Strand cDNA Synthesis** on page 3. Otherwise, store the RNA at –80°C.

First-Strand cDNA Synthesis

Introduction

After you have isolated RNA and checked the quality of your RNA preparation, you are ready to synthesize cDNA.

Before Starting

The following items are supplied by the user:

- 10–40 µg total RNA or 0.4–2 µg mRNA
- Fluorescently labeled dCTP *or* dUTP
- 0.1 M NaOH
- 0.1 M HCl
- Incubator, water bath, or thermal cycler set at 46°C and 70°C
- Ice
- 0.5-ml or 1.5-ml RNase-free microcentrifuge tubes

The following items are supplied in the kit:

- Anchored Oligo(dT)$_{20}$ primer
- Random hexamers (for mRNA starting material only)
- dNTP Mix for Labeled dCTP *or* dNTP Mix for Labeled dUTP
- 5X First-Strand Buffer
- 0.1 M DTT
- RNaseOUT™
- SuperScript™ III RT
- DEPC-treated water
- Control HeLa RNA; optional, see page viii

Important

Fluorescent nucleotides are sensitive to photobleaching. When preparing the reaction, be careful to minimize exposure of the fluorescent nucleotides and labeled DNA to light. We recommend that you wrap reaction tubes in foil to protect against light exposure.

Note

RNaseOUT™ Recombinant RNase Inhibitor has been included in the system to safeguard against degradation of target RNA due to ribonuclease contamination of the RNA preparation.

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First-Strand cDNA Synthesis, continued

First-Strand cDNA Synthesis Reaction

The following procedure is designed to convert 10–40 µg of total RNA or 0.4–2 µg of mRNA into first-strand cDNA. Lower amounts of starting material may be used, but may result in lower hybridization signals.

Note: Volume information is provided for reactions using CyDyes™ and Alexa-Fluor® dyes. If you are using dyes from another manufacturer, adjust the volume of template and labeled nucleotide based on the manufacturers' recommended concentration of labeled nucleotide and a final reaction volume of 30 µl..

If you are setting up a control reaction (recommended for first-time users), use 10 µl of the Control HeLa RNA supplied in the kit (1 µg/µl).

1. Mix and briefly centrifuge each component before use.
2. In a 1.5- or 0.5-ml RNase-free tube, add the following:

Component	Volume	
	CyDyes™	Alexa-Fluor®
10–40 µg total RNA or 0.4–2 µg mRNA	≤11 µl	≤8 µl
Anchored Oligo(dT)$_{20}$ Primer (2.5 µg/µl)	2 µl	2 µl
Random hexamers (only if using mRNA)	1 µl*	1 µl*
DEPC-treated water	to 13 µl	to 10 µl

*For mRNA, use **both** anchored oligo(dT)$_{20}$ and random hexamers. For total RNA, use **only** 2 µl of anchored oligo(dT)$_{20}$.

3. Incubate tube at 70°C for 10 minutes, and then place on ice for at least 1 minute.
4. Add the following to the tube on ice:

Component	Volume	
	CyDyes™	Alexa-Fluor®
5X First-Strand buffer	6 µl	6 µl
0.1 M DTT	3 µl	3 µl
dNTP Mix for labeled dCTP *or* dNTP Mix for labeled dUTP*	2 µl	2 µl
RNaseOUT™ (40 U/µl)	1 µl	1 µl
Labeled dCTP *or* Labeled dUTP (1 mM)	3 µl	6 µl
SuperScript™ III RT (400 U/µl)	2 µl	2 µl
Final Volume	30 µl	30 µl

*Select the appropriate dNTP mix for the labeled dNTP you are using.

5. Mix gently and collect the contents of each tube by brief centrifugation. **Note:** After addition of the labeled nucleotides, be careful to minimize exposure of the tube to light.
6. Incubate tube at 46°C in the dark for 3 hours. **Note:** A 2-hour incubation is sufficient for generating high-quality labeled cDNA with high levels of picomole incorporation; however, a 3-hour incubation will result 10–20% greater incorporation of labeled nucleotides and more full-length cDNA.

After incubation, proceed directly to **Alkaline Hydrolysis and Neutralization** on the next page.

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First-Strand cDNA Synthesis, continued

Hydrolysis and Neutralization

After cDNA synthesis, above, immediately perform the following hydrolysis reaction to degrade the original RNA:

1. Add 15 µl of 0.1 M NaOH to each reaction tube from Step 6, previous page. Mix thoroughly.
2. Incubate tube at 70° C for 30 minutes.
3. Add 15 µl of 0.1 M HCl to neutralize the pH and mix gently.

Proceed to **Purifying the Labeled cDNA** on the following page.

Purifying the Labeled cDNA

Introduction

In this step, you purifiy the labeled cDNA to remove any unincorporated nucleotides.

Cat nos. L1015-01 and L1015-02 include a Purification Module developed for use with the system. Follow the procedure below to purify your labeled cDNA.

Cat no. L1015-03 does not include a Purification Module. Use your preferred method of cDNA purification instead of the following procedure, and then continue to hybridization.

Before Starting

The following items are supplied by the user:

- Microcentrifuge

The following items are supplied in the Purification Module (Cat nos. L1015-01 and L1015-02):

- DEPC-treated water
- Purification columns pre-inserted into collection tubes
- Amber collection tubes
- Loading Buffer **plus isopropanol** (see page vi for preparation)
- Wash Buffer **plus ethanol** (see page vi for preparation)

Purification Procedure

Use the following procedure to purify the cDNA using the components of the Purification Module (Cat nos. L1015-01 and L1015-02). **Note:** Before starting the procedure, be sure to add isopropanol to the Loading Buffer supplied in the kit and ethanol to the Wash Buffer supplied in the kit as described on page vi.

1. Add 40 µl of DEPC-treated water to the reaction tube from **Hydrolysis and Neutralization**, Step 3, page 5, for a final volume of 100 µl.
2. Add 500 µl of Loading Buffer prepared as directed on page vi to the tube. Mix well by vortexing.
3. Each Purification Column is pre-inserted into a collection tube. Load the cDNA/buffer solution directly onto the Purification Column.
4. Centrifuge at 14,000 × *g* at room temperature in a microcentrifuge for 1 minute. Remove the collection tube and discard the flow-through.
5. Place the Purification Column in the same collection tube and add 700 µl of Wash Buffer prepared as directed on page vi to the column.
6. Centrifuge at 14,000 × *g* at room temperature for 2 minutes. Remove the collection tube and discard the flow-through.
7. Place the Purification Column in the same collection tube and centrifuge at 14,000 × *g* at room temperature for 5 minutes. Remove the collection tube and discard the flow-through.
8. Place the Purification Column onto a new **amber** collection tube (supplied in the kit).
9. Add 70 µl of DEPC-treated water to the center of the Purification Column and incubate at room temperature for 1 minute. **Note:** For lower amounts of sample (e.g., starting from <10 µg total RNA), the elution volume can be increased to 100 µl to maximize recovery.
10. Centrifuge at 14,000 × *g* at room temperature for 2 minutes to collect your purified labeled cDNA. **The eluate contains your purified labeled cDNA.**

The sample can stored at –20° C for up to one week prior to hybridization. Avoid freeze/thawing. To determine the efficiency of the labeling reaction, proceed to **Assessing Labeling Efficiency** (page 8).

Hybridization

Hybridization

After purification, you are ready to use the labeled cDNA in any application of choice, including glass microarray hybridization. Follow the preparation and hybridization instructions for your specific application.

Appendix

Assessing Labeling Efficiency

Introduction

You can use the following procedure and formulas to measure the amount of Alexa Fluor®-labeled and CyDye™-labeled cDNA and determine the efficiency of the reaction. The expected amounts of labeled cDNA and incorporated nucleotides using the Control HeLa RNA are noted below.

Absorption Wavelengths and Baselines

The following table shows the absorbance and baseline wavelengths for CyDye™-labeled and Alexa Fluor®-labeled nucleotides:

Label	Absorbance Wavelength	Baseline Wavelength
Alexa Fluor® 555 or Cy3™	550 nm	650 nm
Alexa Fluor® 647 or Cy5™	650 nm	750 nm

Calculating the Amount of Labeled cDNA and Incorporated Nucleotides

To measure the sample:

1. Transfer the **undiluted** sample from Step 10, page 6, into a clean cuvette, and scan at 240–800 nm using a UV/visible spectrophotometer. If you are using a 100-µl cuvette, transfer the entire sample; for smaller cuvettes, transfer an appropriate amount of sample.

   Important: Be sure to blank the spectrophotometer using DEPC-treated water before the reading.

   Note: The labeled cDNA must be purified as described on page 6 before scanning, as any residual unincorporated labeled nucleotides will interfere with the detection of labeled cDNA.

2. Transfer the sample back into the collection tube for storage.

cDNA Yield

Calculate the yield of labeled cDNA using the formula below:

Labeled cDNA (ng) = $A_{260} \times 37$ ng/µl × elution volume (µl)

The amount of cDNA generated from the Control HeLa RNA should be >500 ng. If it is <500 ng, see **Troubleshooting** on page 10.

Labeled Nucleotide Incorporation

Calculate the amount of incorporated labeled nucleotides using a formula below:

Alexa Fluor® 555 (pmole) = $(A_{550}–A_{650})/0.15$ × elution volume (µl)

Alexa Fluor® 647 (pmole) = $(A_{650}–A_{750})/0.24$ × elution volume (µl)

Cy3™ (pmole) = $(A_{550}–A_{650})/0.15$ × elution volume (µl)

Cy5™ (pmole) = $(A_{650}–A_{750})/0.25$ × elution volume (µl)

Control Reaction

The amount of incorporated Alexa Fluor®-labeled nucleotide or CyDye™-labeled nucleotide from the Control HeLa RNA should be >25 picomoles. If it is <20 picomoles, see **Troubleshooting** on page 10.

Determining cDNA Yield Using TCA Precipitation

Introduction

Instructions are provided below to calculate the yield of your first-strand synthesis reaction using TCA precipitation.

Before Starting

Have the following items on hand before starting:

- [α-^{32}P]dCTP
- Yeast tRNA (see page 11 for ordering information)
- 20 mM EDTA
- Glass fiber filters (Fisher Catalog no. 1822-914)
- Heat lamp
- 5% trichloroacetic acid (TCA)
- 10% TCA containing 1% sodium pyrophosphate
- Scintillation counter
- Sterile microcentrifuge tube

Procedure

1. Prepare a first-strand synthesis reaction as described on page 4. Add 1 µl of [α-^{32}P]dCTP (10 mCi/ml, 3,000 mCi/mmol) to the listed components in Step 4.
2. Add 2 µl of the radio-labeled first-strand reaction mix from Step 5, page 4, to a sterile microcentrifuge tube containing 43 µl of 20 mM EDTA (pH 7.5) and 5 µl of yeast tRNA (5 µg). Mix well by vortexing.
3. Spot two 10-µl aliquots from the tube onto separate glass fiber filters.
4. Dry the filters under a heat lamp.
5. Set one filter aside. This will be used to determine the specific activity of dCTP in the reaction.
6. Wash the second filter once in ice-cold 10% TCA containing 1% sodium pyrophosphate for 10 minutes at room temperature on a rotary shaker.
7. Wash the filter twice in 5% TCA for 10 minutes.
8. Wash the filter with 95% ethanol for 10 minutes at room temperature. This filter will be used to determine the yield of the first-strand cDNA.
9. Count both filters using a standard scintillation counter.

Calculating the Yield

Calculate first-strand synthesis yield as follows:

$$\text{Specific activity (cpm/pmole dCTP)} = \frac{\text{cpm of unwashed filter}}{\text{200 pmole dCTP}}$$

$$\text{Amount of cDNA (µg)} = \frac{\text{cpm of washed filter} \times 5 \times 15 \times \text{4 pmole dNTP/pmole dCTP}}{\text{Specific activity} \times \text{3,030 pmole dNTP/µg cDNA}}$$

$$\text{Yield} = \frac{\text{Amount of cDNA (µg)} \times 100}{\text{Amount of mRNA used (µg)*}}$$

If the yield is low, see **Troubleshooting** on page 10.

*If you are using total RNA as your starting material, the mRNA will be 1–2% of total RNA.

Troubleshooting

Problem	Cause	Solution
28S and 18S bands are not observed after isolation of total RNA and agarose gel electrophoresis	Too little RNA loaded on the gel	Be sure to load at least 250 ng of RNA for analysis.
	RNA is degraded due to RNase activity	Follow the guidelines on page 1 to avoid RNase contamination. Use a fresh sample for RNA isolation.
28S band is diminished or low molecular weight RNA appears in the gel	RNA is degraded	Follow the guidelines on page 1 to avoid RNase contamination. Use a fresh sample for RNA isolation.
Yield of cDNA from the first-strand synthesis reaction is low	Temperature too high during cDNA synthesis	Perform the cDNA synthesis at 46° C.
	Incorrect reaction conditions used	Verify that all reaction components are included in the reaction and use reagents provided in the system. Verify the reaction conditions using the Control HeLa RNA provided in the kit.
	Concentration of template RNA is too low	Increase the concentration of template RNA. Use at least 10 µg of total RNA or 0.4 µg of mRNA.
	Poor quality RNA used or RNA is degraded	Check the quality of your RNA preparation (see page 2). If RNA is degraded, use fresh RNA.
	RNase contamination	Use the RNaseOUT™ included in the kit to prevent RNA degradation.
	RT inhibitors are present in your RNA sample	Inhibitors of RT include SDS, EDTA, guanidinium chloride, formamide, sodium phosphate and spermidine (Gerard, 1994). Test for the presence of inhibitors by mixing 1 µg of Control HeLa RNA with 25 µg total RNA or 1 µg mRNA and compare the yields of first-strand synthesis.
	Improper storage of SuperScript™ III RT	Store the enzyme at -20°C.
	Reagents were not properly mixed before use.	Repeat the procedure, being careful to briefly vortex and centrifuge each reagent before use.
Yield of labeled cDNA is low	cDNA has been lost in the purification step	Measure the amount of labeled cDNA produced by the Control RNA before and after purification. Follow the purification procedure without modifications.
	Low amount of starting material	For lower amounts of starting material (e.g., <10 µg total RNA), increase the elution volume in the purification step to 100 µl to maximize recovery.
Amount of incorporated labeled nucleotides in the control reaction is low and/or fluorescence of labeled cDNA is low	Reaction tubes have been exposed to light	Avoid direct exposure of the labeling reaction to light. Use the amber tube provided in the kit for collection of the final product.
	Inefficient labeling due to improper purification	Follow all purification steps carefully and without modification.
	Starting amount of RNA is too low	Increase the amount of starting RNA

Accessory Products

Additional Products

Many of the reagents in the SuperScript™ Direct cDNA Labeling System, as well as additional reagents that may be used with this system, are available separately from Invitrogen. Ordering information is provided below.

Product	Quantity	Catalog no.
Alexa Fluor® 647-aha-dUTP	25 µl	A32763
Alexa Fluor® 555-aha-dUTP	25 µl	A32762
RNase *AWAY*™ Reagent	250 ml	10328-011
TRIzol® Reagent	100 ml	15596-026
	200 ml	15596-018
Micro-to-Midi Total RNA Purification System	50 reactions	12183-018
Micro-FastTrack™ 2.0 mRNA Isolation Kit	20 reactions	K1520-02
FastTrack® 2.0 mRNA Isolation Kit	6 reactions	K1593-02
	18 reactions	K1593-03
RNaseOUT™ Recombinant Ribonuclease Inhibitor	5000 units	10777-019
Yeast tRNA	25 mg	15401-011
	50 mg	15401-029
Human Cot-1 DNA®	500 µg	15279-011
Mouse Cot-1 DNA®	500 µg	18440-016
Random primers	9 A_{260} units	48190-011
UltraPure™ DEPC-treated water	4 × 1.25 ml	10813-012
UltraPure™ 10% SDS solution	4 × 100 ml	15553-027
UltraPure™ 20X SSC	1 L	15557-044
UltraPure™ 20x SSPE	1 L	15591-043

Purchaser Notification

Limited Use Label License No. 18: RNaseOUT™ Ribonuclease Inhibitor

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Corporate Headquarters:
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail: tech_service@invitrogen.com

Japanese Headquarters:
Invitrogen Japan K.K.
Nihonbashi Hama-Cho Park Bldg. 4F
2-35-4, Hama-Cho, Nihonbashi
Tel: 81 3 3663 7972
Fax: 81 3 3663 8242
E-mail: jpinfo@invitrogen.com

European Headquarters:
Invitrogen Ltd
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
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