# BioModule™ Microarray Unit

## For direct or indirect labeling of cDNA and hybridization to microarrays

**Catalog nos. WFGE03 and WFGE04**

**Version B**
04 January 2011
*25-0886*

User Manual

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# Contents and Storage

## Types of Products

This manual is supplied with the following products:

| Product | Catalog no. |
|---|---|
| BioModule™ Microarray Unit | |
| *with Indirect cDNA Labeling* | WFGE03 |
| *with Direct cDNA Labeling* | WFGE04 |

## Number of Boxes

The number of boxes provided with each unit is listed below.

| Components | WFGE03 | WFGE04 |
|---|---|---|
| SuperScript™ Plus Indirect cDNA Labeling System | | |
| *Core Module* | 1 | — |
| *Dye Module* | 1 | |
| *Purification Module* | 1 | |
| SuperScript™ Plus Direct cDNA Labeling System | | |
| *Core Module* | — | 1 |
| *Dye Module* | | 1 |
| *Purification Module* | | 1 |
| DNase I, amplification grade | 2 | 2 |
| PureLink™ Micro-to-Midi Total RNA Purification Kit | 1 | 1 |
| TRIzol® Reagent | 1 | 1 |
| UltraPure™ Formamide | 1 | 1 |
| BioModule™ Microarray Hybridization Solutions | 1 | 1 |

## Shipping and Storage

The shipping conditions for each component are listed below. Upon receipt, store the components as described.

| Component | Shipping | Storage |
|---|---|---|
| TRIzol® Reagent | Room temp. | 2–8ºC |
| PureLink™ Micro-to-Midi Total RNA Purification System | Room temp. | Room temp. |
| DNase I, amplification grade | Dry ice | –20ºC in a non-frost free freezer |
| SuperScript™ Plus Indirect cDNA Labeling System | | |
| *Core Module* | Dry ice | –20ºC |
| *Dye Module* | Dry ice | –20ºC |
| *Purification Module* | Room temp. | Room temp. |
| SuperScript™ Plus Direct cDNA Labeling System | | |
| *Core Module* | Dry ice | –20ºC |
| *Dye Module* | Dry ice | –20ºC |
| *Purification Module* | Room temp. | Room temp. |
| BioModule™ Microarray Hybridization Solutions | Room temp. | 2–8ºC |
| UltraPure™ Formamide | Dry ice | –20ºC |

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# Contents and Storage, continued

**TRIzol® Reagent**

This unit includes 100 ml of TRIzol® Reagent, which is sufficient for ~100 isolations.

**PureLink™ Micro-to-Midi Total RNA Purification**

The components provided with the PureLink™ Micro-to-Midi Total RNA Purification System are described below. Sufficient reagents are provided to perform 50 purifications.

| Item | Amount |
|---|---|
| RNA Lysis Solution | 125 ml |
| Wash Buffer I | 50 ml |
| Wash Buffer II | 15 ml |
| RNase-Free Water | 15 ml |
| RNA Spin Cartridges, with collection tubes | 50 |
| RNA Wash Tubes | 50 |
| RNA Recovery Tubes | 50 |

**DNase I, Amplification Grade**

Two boxes of DNase I, Amplification Grade, are provided with each unit. Components are listed below. Sufficient reagents are provided for 40 on-column DNase I treatments.

| Item | Amount |
|---|---|
| DNase I, Amp Grade | 2 × 100 µl |
| 10X DNase I Reaction Buffer | 2 × 100 µl |
| 25 mM EDTA (pH 8.0) | 2 × 100 µl |

**10X DNase I Reaction Buffer**

10X DNase I Reaction Buffer contains 200 mM Tris-HCl (pH 8.4), 20 mM $MgCl_2$, and 500 mM KCl.

**Microarray Hybridization Solutions**

The BioModule™ Microarray Hybridization Solutions are described below. Formulations are proprietary. Sufficient reagents are provided to perform 30 hybridizations.

| Item | Components/Concentration | Amount |
|---|---|---|
| Pre-Hybridization Solution | Combine with Buffer WB and formamide | 129.5 ml |
| Hybridization Solution | Combine with Buffer WB and formamide | 720 µl |
| Buffer WA | — | 2 × 240 ml |
| Buffer WB | — | 85 ml |

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### UltraPure™ Formamide

This unit includes 500 g of UltraPure™ Formamide in liquid form, which must be added to the Hybridization Solution before use.

### SuperScript™ Plus Indirect cDNA Labeling System

The SuperScript™ Plus Indirect cDNA Labeling System is included with the BioModule™ Microarray Unit with Indirect cDNA labeling (Catalog no. WFGE03). Sufficient reagents are provided to perform 30 labeling reactions.

| Item | Components/Concentration | 30 Rxns |
|---|---|---|
| *Core Module* | | |
| SuperScript™ III Reverse Transcriptase | 400 U/µl | 60 µl |
| 5X First-Strand Buffer | 250 mM Tris-HCl (pH 8.3, room temp), 375 mM KCl, 15 mM $MgCl_2$ | 200 µl |
| Dithiothreitol (DTT) | 0.1 M DTT in water | 250 µl |
| dNTP Mix | dATP, dGTP, dCTP, dTTP, one aminoallyl-modified nucleotide, and one aminohexyl-modified nucleotide | 45 µl |
| 2X Coupling Buffer | — | 300 µl |
| Anchored Oligo$(dT)_{20}$ primer | 2.5 µg/µl in DEPC-treated water | 60 µl |
| Random hexamer primers | 0.5 µg/µl in DEPC-treated water | 30 µl |
| DMSO | — | 750 µl |
| RNaseOUT™ | 40 U/µl | 30 µl |
| DEPC-treated Water | — | 6 ml |
| Control HeLa RNA | 1 µg/µl | 20 µl |
| *Dye Module* | | |
| Alexa Fluor® 555 Reactive Dye Pack | 60 µg dried-down dye per vial | 3 × 5 vials |
| Alexa Fluor® 647 Reactive Dye Pack | 60 µg dried-down dye per vial | 3 × 5 vials |
| *Purification Module* | | |
| Low-Elution Volume Spin Cartridges | Pre-inserted into collection tubes | 6 × 11 columns |
| Binding Buffer | Must be combined with 100% isopropanol to create final buffer; see page 14 | 2 × 18 ml |
| Wash Buffer | Must be combined with 100% ethanol to create final buffer; see page 14 | 2 × 5 ml |
| Amber collection tubes | — | 6 × 11 tubes |

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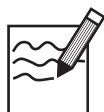
# Contents and Storage, continued

**SuperScript™ Plus Direct cDNA Labeling System**

The SuperScript™ Plus Direct cDNA Labeling System is included with the BioModule™ Microarray Unit with Direct cDNA labeling (Catalog no. WFGE04). Sufficient reagents are provided to perform 30 labeling reactions.

| Item | Components/Concentration | 30 Rxns |
|---|---|---|
| ***Core Module*** | | |
| SuperScript™ III Reverse Transcriptase | 400 U/µl | 60 µl |
| 5X First-Strand Buffer | 250 mM Tris-HCl (pH 8.3, room temp), 375 mM KCl, 15 mM $MgCl_2$ | 200 µl |
| Dithiothreitol (DTT) | 0.1 M DTT in water | 250 µl |
| Anchored Oligo$(dT)_{20}$ primer | 2.5 µg/µl in DEPC-treated water | 60 µl |
| Random hexamer primers | 0.5 µg/µl in DEPC-treated water | 30 µl |
| RNaseOUT™ | 40 U/µl | 30 µl |
| DEPC-treated Water | — | 2 × 2 ml |
| Control HeLa RNA | 1 µg/µl | 20 µl |
| ***Nucleotide Module*** | | |
| 10X Nucleotide Mix with Alexa Fluor® 555-aha-dUTP | Mixture of dCTP, dGTP, dATP, dTTP, and labeled dUTP in 10 mM Tris (pH 8.0), 1 mM EDTA | 3 × 15 µl |
| 10X Nucleotide Mix with Alexa Fluor® 647-aha-dUTP | Mixture of dCTP, dGTP, dATP, dTTP, and labeled dUTP in 10 mM Tris (pH 8.0), 1 mM EDTA | 3 × 15 µl |
| ***Purification Module*** | | |
| Low-Elution Volume Spin Cartridges | Pre-inserted into collection tubes | 3 × 11 columns |
| Binding Buffer | Must be combined with 100% isopropanol to create final buffer; see page 14 | 18 ml |
| Wash Buffer | Must be combined with 100% ethanol to create final buffer; see page 14 | 5 ml |
| Amber collection tubes | — | 3 × 11 tubes |



**Note**

- Some reagents in each unit may be provided in excess of the amount needed.
- Some of the individual products provided with each unit may include documentation describing general use. **We recommend following the protocols in this manual when using the BioModule™ Microarray Unit, for optimal results.**

# Introduction

## Overview

### Introduction

The BioModule™ Microarray Units provide qualified reagents and validated protocols for fluorescent labeling of cDNA synthesized from purified total RNA, and subsequent hybridization of the labeled cDNA to DNA microarrays for gene expression profiling (De Risi *et al.*, 1996; Eisen & Brown, 1999).

The **Indirect cDNA Labeling Unit** includes the SuperScript™ Plus Indirect cDNA Labeling System for generating labeled cDNA using aminoallyl- and aminohexyl-modified nucleotides and either Alexa Fluor® 555 succinimidyl ester or Alexa Fluor® 647 succinimidyl ester.

The **Direct cDNA Labeling Unit** includes the SuperScript™ Plus Direct cDNA Labeling System for generating labeled cDNA using nucleotide mixes that contain Alexa Fluor® 555-aha-dUTP or Alexa Fluor® 647-aha-dUTP.

Both units include TRIzol® Reagent and the PureLink™ Micro-to-Midi Total RNA Purification Kit for high-quality, rapid purification of total RNA, and SuperScript™ III Reverse Transcriptase (RT) for high-temperature synthesis of first-strand cDNA from 5–20 µg of total RNA. Both units also include optimized microarray hybridization buffers that are compatible for use with Corning® GAPS, GAPS II, and UltraGAPS™ slides.

### Summary of Components

Each BioModule™ Microarray Unit includes:

- TRIzol® Reagent and the PureLink™ Micro-to-Midi Total RNA Purification System, for isolating total RNA from mammalian cells, fresh and frozen tissues, whole blood, and liquid samples.
- Deoxyribonuclease I (DNase I), Amplification Grade, for eliminating DNA during RNA purification procedures
- SuperScript™ III Reverse Transcriptase for high-temperature cDNA synthesis, ensuring high specificity and high yields of cDNA as well as more full-length cDNA
- 5X First-Strand Buffer and anchored oligo$(dT)_{20}$ primer for cDNA synthesis
- RNaseOUT™ Recombinant Ribonuclease Inhibitor, to safeguard against the degradation of target RNA due to ribonuclease contamination.
- Alexa Fluor®-labeled nucleotides ***or*** amine-modified nucleotides and Alexa Fluor® dyes, for labeling cDNA
- Spin cartridges and buffers for purifying the labeled cDNA
- Optimized array hybridization and wash buffers

For more information about each reagent, see page 4.

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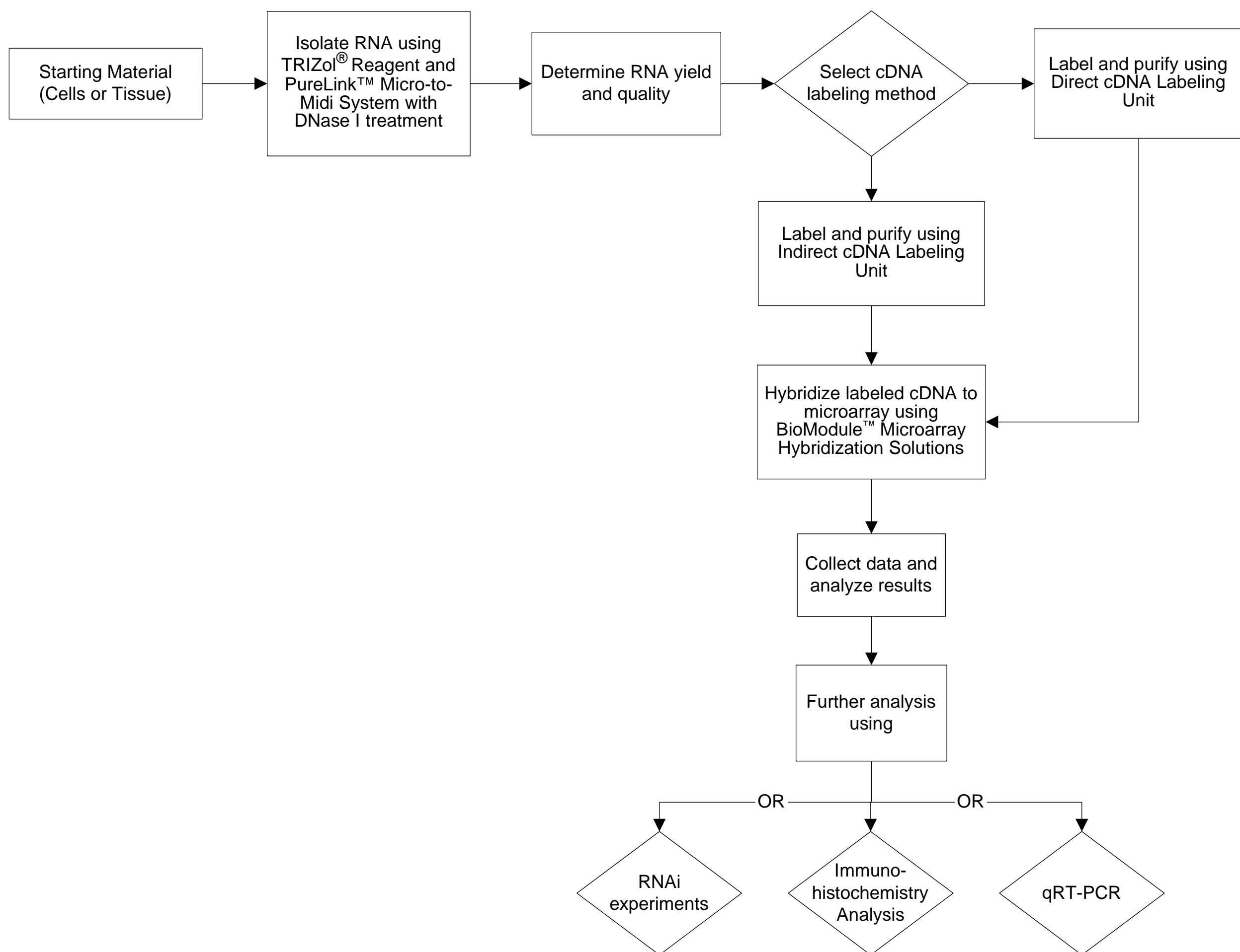
## Labeling and Hybridization Workflow Overview

To use the BioModule™ Microarray Unit, you will:

- Purify total RNA from lysate using TRIzol® Reagent and the PureLink™ Micro-to-Midi Total RNA Purification System, and treat with DNase I
- Determine RNA quality and yield
- Synthesize and label cDNA using indirect or direct labeling
- Purify the labeled cDNA
- Hybridize the labeled cDNA to microarrays
- Analyze the results

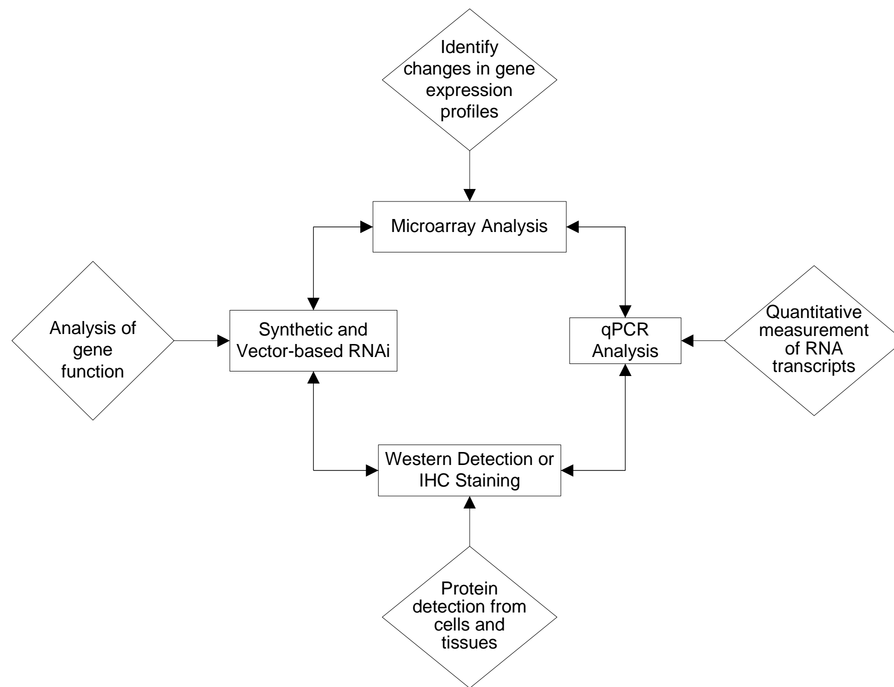


# BioModule™ System Overview

## BioModule™ Units for Gene Expression Profiling

The BioModule™ Microarray Unit is one of several BioModule™ Units available from Invitrogen for gene expression profiling. Each BioModule™ Unit for gene expression profiling includes high-quality reagents and validated protocols with relevant controls for each step of the workflow (see below). Each unit is designed to provide an integrated workflow that allows you to perform various steps seamlessly during expression analysis.

Gene expression profiling comprises multiple steps employing various technologies such as microarray analysis or quantitative RT-PCR (qRT-PCR) for analysis at the nucleic acid level; western immunodetection and immunohistochemistry for analysis at the protein level; and RNAi for functional analysis.



# Description of Components

**Introduction**

This section describes the major components included with each BioModule™ Microarray Unit.

**TRIzol® Reagent**

TRIzol® Reagent is a ready-to-use reagent for the isolation of total RNA from cells and tissues. The reagent is a mono-phasic solution of phenol and guanidine isothiocyanate, and is derived from the RNA isolation method developed by Chomczynski and Sacchi (Chomczynski & Sacchi, 1987).

During sample homogenization or lysis, TRIzol® Reagent maintains the integrity of the RNA while disrupting cells and dissolving cell components. The isolated RNA can then be separated from the other organic material with the addition of chloroform followed by centrifugation. Ethanol is added, and the solution is transferred to the PureLink™ Micro-to-Midi Total RNA Purification System for final purification.

**PureLink™ Micro-to-Midi Total RNA Purification System**

The PureLink™ Micro-to-Midi Total RNA Purification System is suitable for isolating total RNA from animal and plant cells and fresh and frozen tissues.

Using the system, RNA from lysate prepared with TRIzol® Reagent is passed through a spin cartridge that binds the RNA to a silica-based membrane. Impurities are removed by washing, and the purified total RNA is eluted in water.

**DNase I, Amplification Grade**

DNase I, Amplification Grade, digests single-and double-stranded DNA. It is used to remove DNA from the isolated total RNA prior to cDNA synthesis.

**Alexa Fluor® 555 and Alexa Fluor® 647 Reactive Dyes**

The Alexa Fluor® 555 and Alexa Fluor® 647 dyes included with the Indirect cDNA Labeling Unit (Catalog no. WFGE03) are compatible with commonly used microarray scanners, and provide greater signal correlation ($R^2$) values than the spectrally similar Cy™3 and Cy™5 dye pair, improving the resolution of two-color microarray gene expression assays. The exceptionally bright Alexa Fluor® dyes are also insensitive to pH and are highly water-soluble.

**Alexa Fluor® 555 and Alexa Fluor® 647-labeled Nucleotides**

The labeled 5-aminohexylacrylamido-dUTP (aha-dUTP) nucleotides included with the Direct cDNA Labeling Unit (Catalog no. WFGE04) are modified with a unique hexylacrylamide linker, which serves as a spacer between the nucleotide and the dye. This spacer reduces interactions between the nucleotide and the dye, resulting in brighter conjugates.

The Alexa Fluor® 555 and Alexa Fluor® 647 dyes used to label the nucleotides are compatible with commonly used microarray scanners, and provide greater signal correlation ($R^2$) values than the spectrally similar Cy™3 and Cy™5 dye pair, improving the resolution of two-color microarray gene expression assays. The exceptionally bright Alexa Fluor® dyes are also insensitive to pH and are highly water-soluble.

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## Description of Components, continued

**SuperScript™ III Reverse Transcriptase**

SuperScript™ III Reverse Transcriptase is a version of M-MLV RT that has been engineered to reduce RNase H activity and provide increased thermal stability during first-strand cDNA synthesis (Gerard *et al.*, 1986; Kotewicz *et al.*, 1985). The enzyme can be used at a temperature range of 42–60°C, providing increased specificity, higher yields of cDNA, and more full-length product than other reverse transcriptases. Because SuperScript™ III RT is not significantly inhibited by ribosomal and transfer RNA, it can be used to synthesize cDNA from total RNA.

The SuperScript™ III RT in these units is provided at an optimal concentration and used at an optimal temperature for incorporating amino-modified or fluorescently 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, and 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.

**RNaseOUT™**

RNaseOUT™ Recombinant Ribonuclease Inhibitor is an RNase inhibitor protein that safeguards against the degradation of target RNA.

**Low-Elution Volume Spin Cartridges**

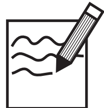
The Low-Elution Volume Spin Cartridges included in the Indirect and Direct cDNA Labeling Units provide a rapid, simple method for the purification of labeled cDNA. The cDNA from the cartridges is highly pure, resulting in more accurate yield and picomole dye incorporation calculations than with other purification methods.

# Methods

## Total RNA Purification

### Introduction

This section provides guidelines and protocols for total RNA purification from animal cells or tissues using TRIzol® Reagent, the PureLink™ Micro-to-Midi Total RNA Purification System, and DNase I, Amplification Grade.



**Note**

The protocols in this section use TRIzol® Reagent, the PureLink™ Micro-to-Midi Total RNA Purification System, and DNase I, Amplification Grade. Have all components from these boxes ready before proceeding.

### Guidelines for Handling RNA

Follow the guidelines below to prevent RNase contamination and to maximize the RNA yield:

- Use disposable, individually wrapped, sterile plastic ware
- Use only sterile, disposable RNAse-free pipette tips and microcentrifuge tubes
- Wear disposable gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin. Change gloves frequently, particularly as the purification protocol progresses from crude extracts to more purified material.
- Always use proper microbiological aseptic techniques when working with RNA
- Use RNase *AWAY*® Reagent (see **Additional Products**, page 40) to remove RNase contamination from work surfaces and non-disposable items such as centrifuges and pipettes used during purification.

### Guidelines for Sample Collection

Use the following guidelines for collecting your samples to minimize RNA degradation prior to RNA purification and to maximize the RNA yield:

- Always wear disposable gloves while handling samples and reagents to prevent RNase contamination.
- Work quickly during sample harvesting, use RNase-free dissection tools and containers (scalpels, dishes, tubes etc.) and work on RNAse-free work surfaces (use RNase *AWAY*® Reagent).
- To purify total RNA from fresh samples, keep fresh cell and tissue samples on ice immediately after harvesting; quickly proceed to adding RNA Lysis solution, sample lysis and homogenizaton.
- To purify total RNA from frozen samples, freeze samples immediately after harvesting in liquid nitrogen or on dry ice. Keep frozen samples at -80° or in liquid nitrogen until proceeding to sample lysis and homogenization.
- **Whole blood:** Process freshly drawn blood immediately, and keep at room temperature until processing.

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- TRIzol® Reagent contains phenol and guanidine isothiocyanate. Toxic in contact with skin and if swallowed. Causes burns. **Always wear a laboratory coat, disposable gloves, and eye protection when handling solutions containing this chemical.** After contact with skin, wash immediately with plenty of detergent and water. Use in a chemical fume hood. Avoid breathing vapor. Do not add bleach or acidic solutions directly to solutions containing guanidine isothiocyanate or sample preparation waste. Guanidine isothiocyanate forms reactive compounds and toxic gases when mixed with bleach or acids.
- Solutions containing ethanol are considered flammable. Use appropriate precautions when using these solutions.

**Important**

- Use polypropylene tubes when working with TRIzol® Reagent. Do not use polystyrene tubes.
- Frozen tissue must remain frozen at –80°C prior to lysis. Cool tubes in dry ice before placing frozen tissue in them. Thawing of frozen tissue prior to lysis may result in RNA degradation and loss of RNA yield.

## Additional Materials Needed

You will need the following additional items not provided with this unit:

- DEPC-treated water
- Chloroform (molecular biology grade)
- 70% ethanol (in RNase-free water)
- 15-ml and 1.5-ml RNase-free polypropylene microcentrifuge tubes (Polypropylene is required for use with TRIzol® Reagent; do not use polystyrene tubes. Round-bottom 15-ml tubes are required for homogenization of tissues using a power homogenizer.)
- Microcentrifuge capable of centrifuging 12,000 × *g*
- Vortex mixer
- For tissue samples: Power homogenizer with a rotating tip that fits a 15-ml tube (*e.g.*, Ultra Turrax® or Polytron® Homogenizer )
- 1.5 ml RNase-free tubes
- RNase-free pipette tips

## Preparing Wash Buffer II with Ethanol

Before using Wash Buffer II from the PureLink™ Micro-to-Midi Total RNA Purification System for the first time, add 60 ml of 96–100% ethanol directly to the bottle. Check the box on the Wash Buffer II label to indicate that ethanol was added.

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## Lysis and Homogenization of Cells

Follow the steps below to prepare lysates from up to 1 × $10^6$ cells:

1. **Adherent cells:** Lift cells from the plate by adding TE and resuspend in culture media. Count the cells, and transfer ≤1 × $10^6$ cells to a 15-ml polypropylene centrifuge tube.

   **Suspension cells:** Transfer ≤1 × $10^6$ cells to a 15-ml polypropylene centrifuge tube.
2. Centrifuge at 2,000 × *g* for 5 minutes at room temperature to pellet the cells.
3. Carefully pipet the supernatant from the tube, leaving no more than 30 µl of supernatant. Take care not to disturb the cell pellet.
4. Immediately add 1 ml of TRIzol® to the tube. Vortex until the cell pellet is completely lysed and no visible particulate matter remains. If necessary, pipet the pellet up and down to disperse.
5. Transfer the lysate to a new, RNase-free 1.5-ml polypropylene microcentrifuge tube.

Proceed to **Total RNA Purification Procedure**, next page.

## Lysis and Homogenization of Tissues

Follow the steps below to prepare lysates from animal tissues. Use a power homogenizer with a rotating tip that fits a 15-ml round-bottom tube.

**Note:** The volume of tissue should not exceed 10% of the volume of TRIzol® Reagent used for homogenization. Keep frozen tissues on dry ice until just prior to homogenization.

1. Add 1 ml of TRIzol® Reagent for every 50–100 mg of tissue to a 15-ml round-bottom polypropylene microcentrifuge tube. Immediately add fresh or frozen tissue to the tube and quickly homogenize the sample using a power homogenizer at a medium setting. Homogenize using short bursts for ~2 minutes. Avoid foaming by keeping the rotating tip submerged in solution while holding the tip against the tube wall. Keep homogenate on ice.
2. Transfer 1-ml aliquots of homogenate to separate 1.5-ml polypropylene microcentrifuge tubes on ice.
3. Centrifuge tubes at 2,000 × *g* for 5 minutes at 5°C to pellet cellular debris.
4. Carefully transfer the supernatant (~800 µl) containing the RNA from each tube to a new, RNase-free 1.5-ml polypropylene tube.
5. Add enough TRIzol® (~200 µl) to the tube to bring the total volume to 1 ml.

Proceed to **Total RNA Purification Procedure**, next page.

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### Total RNA Purification Procedure

Follow the steps below to purify your total RNA sample:

1. Add 0.2 ml of chloroform to each 1.5-ml tube from Step 5 of the lysis and homogenization protocols on the previous page. Mix well by vortexing.
2. Incubate at room temperature for 2 minutes to allow for phase separation.
3. Centrifuge the tube at 12,000 × *g* for 15 minutes at 5°C. After centrifugation, a clear aqueous upper phase and pink organic lower phase should be visible. Some white flocculent material may also be visible in the interface between the phases.
4. Using a pipette, very carefully remove the upper phase (up to 0.5 ml) without disturbing the lower phase or interface. Take care not to remove any flocculent material with the upper phase. (It is better to remove less of the upper phase if there is a danger of disturbing the lower phase or interface.) Transfer the upper phase to a new RNase-free 1.5-ml tube.
5. To the volume of upper-phase solution in the new tube, add an equal volume of 70% ethanol by adding ½ volumes sequentially, and mixing after each addition. Mix carefully to avoid precipitation of RNA due to local concentrations of ethanol.
6. Transfer up to 700 µl of the sample to a PureLink™ RNA Spin Cartridge pre-inserted in a collection tube. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and re-insert the cartridge in the tube.
7. Repeat Step 6 until the entire sample has been processed.
8. Add 350 µl of Wash Buffer I to the spin cartridge. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and re-insert the cartridge in the tube.
9. In a separate RNase-free tube, prepare a DNase I solution by adding:

| | |
|---|---|
| DNase I, Amplification Grade | 5 µl |
| 10X DNase I Reaction Buffer | 5 µl |
| DEPC-treated water | 40 µl |

10. Add the entire volume of DNase I solution directly onto the spin cartridge. Incubate at room temperature for 15 minutes.
11. Add 350 µl of Wash Buffer I to the spin cartridge. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the collection tube.
12. Place the spin cartridge into a clean RNA Wash Tube, provided in the kit.
13. Add 500 µl Wash Buffer II with ethanol (prepared as described on page 7) to the spin cartridge. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and re-insert the cartridge in the tube.
14. Repeat Step 13 once.
15. Centrifuge the spin cartridge at 12,000 × *g* for 1 minute to dry the membrane with attached RNA. Discard the collection tube, and insert the cartridge into an RNA Recovery Tube supplied with the kit.

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# Total RNA Purification, continued

### Total RNA Purification Procedure, continued

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16. To elute the RNA, add 30 µl of RNase-free water to the center of the spin cartridge membrane, and incubate at room temperature for 1 minute.
17. Centrifuge the spin cartridge for 2 minutes at ≥12,000 × *g* to collect the eluate.
18. Remove the recovery tube and add 10 µl of DEPC-treated water to the eluate. Add this solution back onto the spin cartridge membrane. Re-insert the cartridge in the recovery tube, and incubate at room temperature for 1 minute.
19. Centrifuge the spin cartridge for 2 minutes at ≥12,000 × *g* to collect the eluate containing the total RNA.

To determine the quantity and quality of the RNA, see page 11, or store the purified total RNA at –80°C.

# Determining RNA Yield and Quality

## Introduction

After you have purified the total RNA, determine the quantity and quality as described in this section.

## Determining RNA Yield

Total RNA is easily quantitated using the Quant-iT™ RiboGreen® RNA Assay Kit or UV absorbance at 260 nm.

**Quant-iT™ RiboGreen® RNA Assay Kit**

The Quant-iT™ RiboGreen® RNA Assay Kit (catalog no. R-11490; see page 40 for ordering information) provides a rapid, sensitive, and specific method for RNA quantitation with minimal interference from DNA, protein, or other common contaminants that affect UV absorbance readings.

The kit contains a state-of-the-art quantitation reagent and pre-diluted standards for a standard curve. The assay is performed in a microtiter plate format and is designed to be read using a standard fluorescent microplate reader.

**UV Absorbance**

To determine the quantity by UV absorbance:

1. Dilute an aliquot of the total RNA sample in 10 mM Tris-HCl, pH 7.5. Mix well. Transfer to a cuvette (1-cm path length). **Note:** The RNA must be in a neutral pH buffer to accurately measure the UV absorbance.
2. Determine the $A_{260}$ of the solution using a spectrophotometer blanked against 10 mM Tris-HCl, pH 7.5.

Calculate the amount of total RNA using the following formula:

Total RNA (µg) = $A_{260}$ × [40 µg/(1 $A_{260}$ × 1 ml)] × dilution factor × total sample volume (ml)

**Example:**
Total RNA was eluted in water in a total volume of 150 µl. A 40-µl aliquot of the eluate was diluted to 500 µl in 10 mM Tris-HCl, pH 7.5. An $A_{260}$ of 0.188 was obtained. The amount of RNA in the sample is determined as shown below:

Total RNA (µg) = 0.188 × [40 µg/(1 $A_{260}$ × 1 ml)] × 12.5 × 0.15 = 14.1 µg

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# Determining RNA Yield and Quality, continued

## Analyzing RNA Quality

**Bioanalyzer:** RNA quality can be analyzed with an Agilent Bioanalyzer, using a 200 ng/µl dilution of total RNA. Follow the instrument manufacturer's instructions.

**Gel Electrophoresis:** Quality may also be analyzed by agarose/ethidium bromide gel electrophoresis. We recommend using 500 ng of RNA loaded onto a 1.2% E-Gel™ agarose gel (see page 40 for ordering information). For total human RNA using an 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.

# Indirect cDNA Labeling

## Introduction

This section provides protocols for using the SuperScript™ Plus Indirect cDNA Labeling System, included with BioModule™ catalog no. WFGE03.

Using this system, you synthesize first-strand cDNA from total RNA using aminoallyl-modified nucleotides and aminohexyl-modified nucleotides together with other dNTPs in a reaction with SuperScript™ III Reverse Transcriptase. After a purification step to remove unincorporated nucleotides, the amino-modified cDNA is coupled with a monoreactive, N-hydroxysuccinimide (NHS)-ester fluorescent dye—either Alexa Fluor® 555 succinimidyl ester or Alexa Fluor® 647 succinimidyl ester. A final purification step removes any unreacted dye, and the fluorescently labeled cDNA is ready for hybridization to microarrays.

## Additional Materials Needed

In addition to the SuperScript™ Indirect cDNA Labeling System components, you will need the following items:

- Vortex mixer
- Microcentrifuge
- Water baths, heating blocks, or incubator
- Aerosol resistant RNase-free pipette tips
- 1.5-ml RNase-free microcentrifuge tubes
- 1 N NaOH
- 1 N HCl
- 100% Isopropanol
- 100% Ethanol
- 75% Ethanol

## Control Reaction

We recommend performing the labeling procedure using the Control HeLa RNA included in the system to determine the efficiency of the labeling reaction.

## Amount of RNA

This system is optimized for use with 5–20 µg total RNA. Lower amounts of starting material may be used, but may result in lower hybridization signals.

## Alexa Fluor® 555 and Alexa Fluor® 647 Reactive Dyes

The Alexa Fluor® 555 and Alexa Fluor® 647 dyes are compatible with commonly used microarray scanners. The table below shows the excitation and emission maxima and color of each dye:

| Dye | Excitation/Emission (nm) | Color |
|---|---|---|
| Alexa Fluor® 555 | 555/565 | Orange Fluorescent |
| Alexa Fluor® 647 | 650/670 | Far-Red Fluorescent |

*Continued on next page*

### 5X First-Strand Buffer

The 5X First-Strand Buffer includes 250 mM Tris-HCl (pH 8.3 at room temperature), 375 mM KCl, and 15 mM $MgCl_2$.

### Preparing Binding Buffer with Isopropanol

The Binding Buffer supplied with the Purification Module must be mixed with 100% isopropanol prior to use. Add the amount of isopropanol indicated below directly to the bottle of Binding Buffer to create the final buffer. Be sure to mark the appropriate checkbox on the bottle to indicate that you have added the isopropanol.

| | 10-rxn kit | 30-rxn kit |
|---|---|---|
| Binding Buffer | 5.5 ml (entire bottle) | 18.0 ml (entire bottle) |
| 100% Isopropanol | 2.0 ml | 6.5 ml |
| Final Volume | 7.5 ml | 24.5 ml |

Store the Binding Buffer prepared with isopropanol at room temperature.

### Preparing Wash Buffer with Ethanol

The Wash Buffer supplied with the Purification Module must be mixed with 100% ethanol prior to use. Add the amount of ethanol indicated below directly to the bottle of Wash Buffer to create the final buffer. Be sure to mark the appropriate checkbox on the bottle to indicate that you have added the ethanol.

| | 10-rxn kit | 30-rxn kit |
|---|---|---|
| Wash Buffer | 2 ml (entire bottle) | 5 ml (entire bottle) |
| 100% Ethanol | 8 ml | 20 ml |
| Final Volume | 10 ml | 25 ml |

Store the Wash Buffer prepared with ethanol at room temperature.

### Important

- Fluorescent dyes are sensitive to photobleaching. Be careful to minimize exposure of the dye solution to light. The dye coupling reaction must be incubated in the dark.
- DMSO (used to resuspend the dyes) is hygroscopic and will absorb moisture from the air. Water absorbed from the air will react with the NHS ester of the dye and significantly reduce the coupling reaction efficiency. Keep the DMSO supplied in the kit in an amber screw-capped vial at –20°C, and let the vial warm to room temperature before opening to prevent condensation.

*Continued on next page*

## First-Strand cDNA Synthesis

The following procedure is designed to convert 5–20 µg of purified total RNA into first-strand cDNA.

**Note:** 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) in place of the total RNA in Step 2.

1. Mix and briefly centrifuge each component before use.
2. Prepare each reaction as follows in a 1.5-ml RNase-free tube:

| Component | Volume |
|---|---|
| 5–20 µg purified total RNA | X µl |
| Anchored oligo(dT)$_{20}$ primer (2.5 µg/µl) | 2 µl |
| DEPC-treated water | to 18 µl |

3. Incubate tubes at 70°C for 5 minutes, and then place on ice for at least 1 minute.
4. Add the following to each tube on ice:

| Component | Volume |
|---|---|
| 5X First-Strand buffer | 6 µl |
| 0.1 M DTT | 1.5 µl |
| dNTP mix (including amino-modified nucleotides) | 1.5 µl |
| RNaseOUT™ (40 U/µl) | 1 µl |
| SuperScript™ III RT (400 U/µl) | 2 µl |
| Final volume | 30 µl |

5. Mix gently and collect the contents of each tube by brief centrifugation. Incubate tube at 46°C for 2–3 hours. **Note:** A 3-hour incubation results in 20–30% higher cDNA yield than a 2-hour incubation.

After incubation, proceed directly to **Alkaline Hydrolysis and Neutralization**, below.

## Hydrolysis and Neutralization

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

1. Add 15 µl of 1 N NaOH to each reaction tube from Step 5, above. Mix thoroughly.
2. Incubate tube at 70°C for 10 minutes.
3. Add 15 µl of 1 N HCl to neutralize the pH and mix gently.

Proceed to **Purifying the First-Strand cDNA** on the next page.

*Continued on next page*

# Indirect cDNA Labeling, continued

## Purifying the First-Strand cDNA

Use the following procedure to purify the first-strand cDNA.

1. Add 700 µl of Binding Buffer (prepared with isopropanol as described on page 14) to the reaction tube containing the first-strand cDNA from **Hydrolysis and Neutralization**, Step 3, previous page. Vortex briefly to mix.
2. Each Low-Elution Volume Spin Cartridge is preinserted into a collection tube. For multiple reactions, clearly label each collection tube, and then load the cDNA/Binding Buffer solution directly onto the Spin Cartridge.
3. Centrifuge at 3,300 × *g* in a microcentrifuge for 1 minute. Remove the collection tube and discard the flow-through.
4. Place the Spin Cartridge in the same collection tube and add 600 µl of Wash Buffer (prepared with ethanol as described on page 14) to the column.
5. Centrifuge at maximum speed for 30 seconds. Remove the collection tube and discard the flow-through.
6. Place the Spin Cartridge in the same collection tube and centrifuge at maximum speed for 30 seconds to remove any residual Wash Buffer. Remove the collection tube and discard.
7. Place the Spin Cartridge onto a new **amber** collection tube (supplied in the kit).
8. Add 20 µl of DEPC-treated water to the center of the Spin Cartridge and incubate at room temperature for 1 minute.
9. Centrifuge at maximum speed for 1 minute to collect the purified first-strand cDNA in the amber tube. **The eluate contains your purified cDNA.**

Proceed directly to **Coupling the Fluorescent Dye** below.

## Coupling the Fluorescent Dye

Follow the steps below to couple Alexa Fluor® dye to the amino-modified first-strand cDNA. Use only the DMSO provided with this kit.

1. Dry the purified first-strand cDNA from Step 9, above, in a speed vac at medium heat until the volume is reduced to ~3 µl. Be careful not to overdry the sample.
2. Add 5 µl of 2X Coupling Buffer to the tube.
3. Add 2 µl of DMSO directly to a vial of Alexa Fluor® Reactive Dye to resuspend the dye. Vortex thoroughly and then spin briefly to collect the contents.
4. Add the DMSO/dye solution to the tube from Step 2 and vortex to mix thoroughly.
5. Incubate the tube at room temperature **in the dark** for 1–2 hours. The reaction can be stored overnight if necessary.

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

*Continued on next page*

## Purifying the Labeled cDNA

Use the following procedure to purify the fluorescently labeled cDNA.

1. Add 700 µl of Binding Buffer (prepared with isopropanol as described on page 14) to the reaction tube containing the labeled cDNA from **Coupling Procedure**, Step 5, previous page. Vortex briefly to mix.
2. Each Low-Elution Volume Spin Cartridge is preinserted into a collection tube. For multiple reactions, clearly label each collection tube, and then load the cDNA/Binding Buffer solution directly onto the Spin Cartridge.
3. Centrifuge at 3,300 × *g* in a microcentrifuge for 1 minute. Remove the collection tube and discard the flow-through.
4. Place the Spin Cartridge in the same collection tube and add 600 µl of Wash Buffer (prepared with ethanol as described on page 14) to the column.
5. Centrifuge at maximum speed for 30 seconds. Remove the collection tube and discard the flow-through.
6. Place the Spin Cartridge in the same collection tube and centrifuge at maximum speed for 30 seconds to remove any residual Wash Buffer. Remove the collection tube and discard.
7. Place the Spin Cartridge onto a new **amber** collection tube (supplied in the kit).
8. Add 20 µl of DEPC-treated water to the center of the Spin Cartridge and incubate at room temperature for 1 minute.
9. Centrifuge at maximum speed for 1 minute to collect the purified cDNA. **The eluate contains your purified labeled cDNA.**

The sample can be 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 22).

# Direct cDNA Labeling

### Introduction

This section provides protocols for using the SuperScript™ Plus Direct cDNA Labeling System, included with BioModule™ catalog no. WFGE04.

Using this system, you synthesize first-strand cDNA from total RNA using fluorescently labeled nucleotides in a reaction with SuperScript™ III Reverse Transcriptase. 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.

### Additional Materials Needed

In addition to the SuperScript™ Direct cDNA Labeling System components, you will need the following items:

- Vortex mixer
- Microcentrifuge
- Water bath, incubator, or thermal cycler
- Aerosol resistant RNase-free pipette tips
- 0.5-ml or 1.5-ml RNase-free microcentrifuge tubes
- 0.1 N NaOH
- 0.1 N HCl
- 100% Isopropanol
- 100% Ethanol

### Control Reaction

We recommend performing the labeling procedure using the Control HeLa RNA included in the system to determine the efficiency of the labeling reaction.

### Amount of RNA

The kit has been optimized for use with 5–20 µg of total RNA as starting material. Lower amounts of starting material may be used, but may result in lower hybridization signals.

### Alexa Fluor® 555 and Alexa Fluor® 647-labeled Nucleotides

The Alexa Fluor® 555 and Alexa Fluor® 647 dyes used to label the nucleotides are compatible with commonly used microarray scanners. The table below shows the excitation and emission maxima and color of each dye:

| Dye | Excitation/Emission (nm) | Color |
|---|---|---|
| Alexa Fluor® 555 | 555/565 | Orange Fluorescent |
| Alexa Fluor® 647 | 650/670 | Far-Red Fluorescent |

### 5X First-Strand Buffer

The 5X First-Strand Buffer includes 250 mM Tris-HCl (pH 8.3 at room temperature), 375 mM KCl, and 15 mM $MgCl_2$.

*Continued on next page*

### Preparing Binding Buffer with Isopropanol

The Binding Buffer supplied with the Purification Module must be mixed with 100% isopropanol prior to use. Add the amount of isopropanol indicated below directly to the bottle of Binding Buffer to create the final buffer. Be sure to mark the appropriate checkbox on the bottle to indicate that you have added the isopropanol.

| | 10-rxn kit | 30-rxn kit |
|---|---|---|
| Binding Buffer | 5.5 ml (entire bottle) | 18.0 ml (entire bottle) |
| 100% Isopropanol | 2.0 ml | 6.5 ml |
| Final Volume | 7.5 ml | 24.5 ml |

Store the Binding Buffer prepared with isopropanol at room temperature.

### Preparing Wash Buffer with Ethanol

The Wash Buffer supplied with the Purification Module must be mixed with 100% ethanol prior to use. Add the amount of ethanol indicated below directly to the bottle of Wash Buffer to create the final buffer. Be sure to mark the appropriate checkbox on the bottle to indicate that you have added the ethanol.

| | 10-rxn kit | 30-rxn kit |
|---|---|---|
| Wash Buffer | 2 ml (entire bottle) | 5 ml (entire bottle) |
| 100% Ethanol | 8 ml | 20 ml |
| Final Volume | 10 ml | 25 ml |

Store the Wash Buffer prepared with ethanol at room temperature.

**Important**

Fluorescent dyes are sensitive to photobleaching. Be careful to minimize exposure of the labeled nucleotides to light. The dye coupling reaction must be incubated in the dark.

*Continued on next page*

## First-Strand cDNA Synthesis

The following procedure is designed to convert 5–20 µg of total RNA into labeled first-strand cDNA. Lower amounts of starting material may be used, but may result in lower hybridization signals.

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 |
|---|---|
| 5–20 µg total RNA | X µl |
| Anchored oligo(dT)$_{20}$ primer (2.5 µg/µl) | 2 µl |
| DEPC-treated water | to 15 µl |

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 |
|---|---|
| 5X First-Strand buffer | 6 µl |
| 0.1 M DTT | 3 µl |
| 10X Nucleotide Mix with Alexa Fluor® 555-aha-dUTP ***or*** 10X Nucleotide Mix with Alexa Fluor® 647-aha-dUTP | 3 µl |
| RNaseOUT™ (40 U/µl) | 1 µl |
| SuperScript™ III RT (400 U/µl) | 2 µl |
| Final Volume | 30 µl |

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 in 10–20% greater incorporation of labeled nucleotides and more full-length cDNA.

After incubation, proceed directly to **Hydrolysis and Neutralization**, below.

## Hydrolysis and Neutralization

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

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

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

*Continued on next page*

## Purifying the Labeled cDNA

Use the following procedure to purify the labeled cDNA.

1. Add 700 µl of Binding Buffer (prepared with isopropanol as described on page 19) to the reaction tube containing the labeled cDNA from **Hydrolysis and Neutralization**, Step 3, previous page.
2. Each Low-Elution Volume Spin Cartridge is preinserted into a collection tube. For multiple reactions, clearly label each collection tube, and then load the cDNA/Binding Buffer solution directly onto the Spin Cartridge.
3. Centrifuge at 3,300 × *g* in a microcentrifuge for 1 minute. Remove the collection tube and discard the flow-through.
4. Place the Spin Cartridge in the same collection tube and add 600 µl of Wash Buffer (prepared with ethanol as described on page 19) to the column.
5. Centrifuge at maximum speed for 30 seconds. Remove the collection tube and discard the flow-through.
6. Place the Spin Cartridge in the same collection tube and centrifuge at maximum speed for 30 seconds to remove any residual Wash Buffer. Remove the collection tube and discard.
7. Place the Spin Cartridge onto a new **amber** collection tube (supplied in the kit).
8. Add 20 µl of DEPC-treated water to the center of the Spin Cartridge and incubate at room temperature for 1 minute.
9. Centrifuge at maximum speed for 1 minute to collect the purified labeled cDNA. **The eluate contains your purified labeled cDNA.**

The sample can be 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 22).

# Assessing Labeling Efficiency

**Introduction**

You can use UV/visible spectroscopy scanning to measure the amount of labeled cDNA and dye incorporation. The expected amounts using the Control HeLa RNA provided in the kit are shown below.

**Calculating the Results**

To calculate the amount of labeled cDNA using a UV/visible spectrophotometer:

1. Transfer a volume of purified, labeled cDNA to a clean cuvette. Use an appropriate volume for your spectrophotometer. Add DEPC-treated water to the cDNA if you need to increase the volume of the eluate for your spectrophotometer.

   **Note:** The labeled DNA must be purified before scanning, as any unincorporated dye will interfere with the detection of labeled DNA.

2. Blank the spectrophotometer using DEPC-treated water, and then scan the sample at 240–800 nm. Wash each cuvette thoroughly between samples.
3. Calculate the yield of cDNA using the following formula:

   cDNA (ng) = $(A_{260}–A_{320}) \times 37$ ng/µl × volume in µl

4. Calculate the amount of fluorescent dye using the following formulas:

   Alexa Fluor® 555 (pmole) = $(A_{555}–A_{650})/0.15 \times$ volume in µl

   Alexa Fluor® 647 (pmole) = $(A_{650}–A_{750})/0.24 \times$ volume in µl

5. Calculate the base-to-dye ratio using the following formulas:

   Base/dye ratio for Alexa Fluor® 555 =
   $[(A_{260} – A_{320}) – [(A_{555} – A_{650}) \times 0.04]] \times 150{,}000/(A_{555} – A_{650}) \times 8{,}919$

   Base/dye ratio for Alexa Fluor® 647 =
   $[(A_{260} – A_{320}) – [(A_{650} – A_{750}) \times 0]] \times 239{,}000/(A_{650} – A_{750}) \times 8{,}919$

The number of dye molecules per 100 bases is calculated using the formula: 100/(base/dye ratio)

**Expected Amounts Using Control DNA**

If you prepared a control reaction using 10 µg of Control HeLa RNA as starting material, the following amounts are expected.

**Indirect Labeling:**

| Labeled cDNA | Incorporated Dye | Dyes Molecules/100 Bases |
|---|---|---|
| ≥ 250 ng | ≥ 24 pmole | ≥ 2.50 |

**Direct Labeling:**

| Labeled cDNA | Incorporated Labeled Nuc. | Dyes Molecules/100 Bases |
|---|---|---|
| ≥ 400 ng | ≥ 30 pmole | ≥ 1.0 |

If you do not obtain these amounts, see **Troubleshooting** on page 33.

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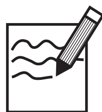
## Assessing Labeling Efficiency, continued

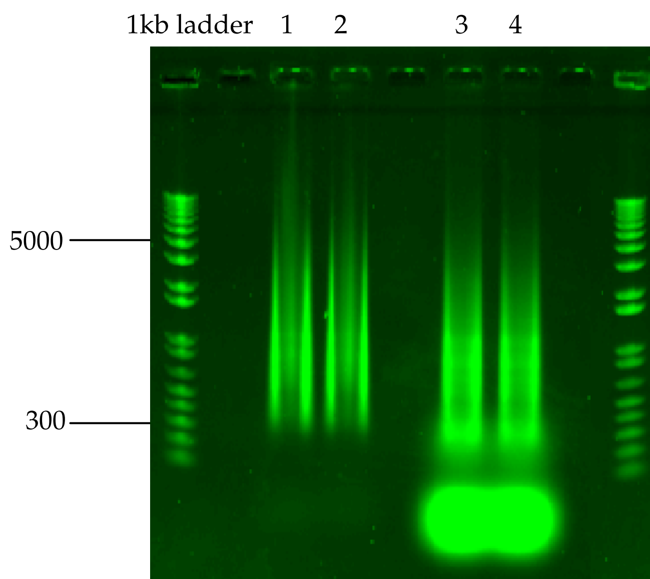


**Note**

Because of the high purity of the cDNA from the Low-Elution Volume Spin Cartridges, the yield and picomole dye incorporation calculations will be more accurate than with other purification methods.

In the 1.2% E-Gel below, Lanes 1 and 2 contain Alexa Fluor® 555-labeled cDNA purified using the Low-Elution Volume Spin Cartridges, and Lanes 3 and 4 contain Alexa Fluor® 555-labeled cDNA purified using columns from another manufacturer. The labeled cDNA appears as smear from 500–5,000 bp.

The large band at the bottom of Lanes 3 and 4 is unincorporated dye that was not removed by the other manufacturer's purification column. Such material would be included in the picomole dye incorporation calculations, resulting in an incorporation level that is higher than theoretically possible.



# Microarray Hybridization

## Introduction

After cDNA labeling and purification, you are ready to hybridize your samples to a glass-slide microarray. This section provides protocols for hybridization using the BioModule™ Microarray Hybridization Solutions.

## Amount of Labeled cDNA

For hybridization, use the entire volume of the labeling reaction from Step 9, page 17 (Indirect Labeling), or Step 9, page 21 (Direct Labeling).

## Glass Slide Surface Chemistries

The BioModule™ Microarray Hybridization Solutions have been developed and optimized for use with Corning® GAPS, GAPS II, and UltraGAPS™ slides.

## Types of Capture Probes

The protocol in this manual produces good results with oligonucleotide probes of approximately 70 bases and longer.

MEEBO (Mouse Exonic Evidence-Based Oligonucleotide) and HEEBO (Human Exonic Evidence-Based Oligonucleotide) probe sets are collections of open source 70-mer oligo probes, largely derived from constitutively expressed exons, allowing interrogation of thousands of genes. These sets are available separately from Invitrogen (see page 40).

## Avoiding Background on Slides

Take care when processing slides to avoid contamination by dust particles or residue from dried droplets of buffers on either side of the glass slide. Any contamination of this sort can cause nonspecific background. To minimize background:

- Make sure that buffers do not air dry on the slides between or after washes.
- **Immediately** centrifuge or use clean, filtered, compressed air to dry the slides after pre-hybridization and washing.
- Never use powdered gloves. Handle slides with forceps if possible, especially during the final wash step.

## Blocking Non-Specific Binding

For customers spotting their own arrays on Corning® GAPS, GAPS II, and UltraGAPS™ slides, chemical blocking with succinic anhydride is not recommended because it can lead to high levels of non-specific background. The pre-hybridization procedure described on page 27 is sufficient to block nonspecific binding of target cDNA to the slide surface. In addition, we do not recommend washing the slides with SDS-containing buffers after printing and prior to processing of the arrays.

## Negative Controls

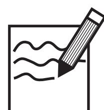
For negative control spots, we recommend using DNA from a species different and distinct from the one studied. Spotting buffer, such as 3X SSC, is not an appropriate negative control because it may produce a higher background.

*Continued on next page*

### Additional Materials Needed

In addition to the components in this unit, you will need the following items:

- Microarray—PCR product or oligonucleotide probes printed on Corning® GAPS, GAPS II, or UltraGAPS™ Slides
- Slide box, or clean, dry slide mailer(s)
- Hybridization chamber (*e.g.*, Corning® Hybridization Chamber, catalog # 2551 or # 40080)
- Raised-edge coverslips (*e.g.*, LifterSlips™, Erie Scientific, catalog # 22x50I-2-4711 or 22x60I-2-4861)
- Hybridization blocking DNA (*e.g.*, Human Cot-1 DNA®, Mouse Cot-1 DNA®, or Salmon-Sperm DNA Solution; see page 40 for ordering information)
- Digital microarray scanner (*e.g.*, the GenePix® 4000B from Molecular Devices) and associated software
- Slide rack
- Wash containers for individual slides (*e.g.*, Coplin jars)
- Wash tanks, capable of completely submerging a slide rack
- Vortex mixer
- Orbital shaker
- Microcentrifuge
- Heat block (95°C)
- Water bath (42°C)
- Temperature-controlled incubator (42°C)
- Tabletop centrifuge with a microtiter plate rotor adapter capable of holding a slide rack or slide holder ***or*** filtered, compressed air
- Lint-free laboratory wipes
- Squirt Bottle
- Aerosol-resistant pipette tips
- 1.5-ml RNase-free microcentrifuge tubes



**Note**

- Prepare the following solutions before use. Solutions should be used within a few days after preparation.
- Warm Buffer WB to 42°C to ensure that any precipitate has dissolved.

### Pre-Hybridization Solution

The Pre-Hybridization Solution is supplied as a concentrate. To prepare for use, add Buffer WB and formamide directly to the bottle, as follows:

| | |
|---|---|
| Pre-Hybridization Solution | 129.5 ml (entire bottle) |
| Buffer WB | 1.75 ml |
| Formamide (molecular biology grade) | 44 ml |

Store at 2–8°C, and heat to 42°C before each use to ensure that any precipitate is completely dissolved.

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### Hybridization Solution

The Hybridization Solution is supplied as a concentrate. To prepare for use, add Buffer WB and formamide directly to the bottle, as follows:

| | |
|---|---|
| Hybridization Solution | 720 µl (entire bottle) |
| Buffer WB | 30 µl |
| Formamide (molecular biology grade) | 750 µl |

Store at 2–8°C, and heat to 42°C before each use to ensure that any precipitate is completely dissolved.

### Wash Solution 1

Prepare 700 ml of Wash Solution 1 to process 3–4 slides. Prepare as follows:

1. Combine the following reagents in an appropriate container, in the order they are listed.

| | |
|---|---|
| De-ionized water | 623 ml |
| Buffer WA | 70 ml |
| Buffer WB | 7 ml |
| Total Volume | 700 ml |

2. Invert gently 3–5 times.

Store Wash Solution 1 at 2–8°C, and heat to 42°C before each use to ensure that any precipitate is completely dissolved.

### Wash Solution 2

Prepare 400 ml of Wash Solution 2 to process 3–4 slides. Prepare as follows:

1. Combine the following reagents in an appropriate container, in the order they are listed.

| | |
|---|---|
| De-ionized water | 394 ml |
| Buffer WA | 2 ml |
| Buffer WB | 4 ml |
| Total Volume | 400 ml |

2. Invert gently 3–5 times.

Keep Wash Solution 2 at room temperature.

### Wash Solution 3

Prepare 1,000 ml of Wash Solution 3 to process 3–4 slides. Prepare as follows:

1. Combine the following reagents in an appropriate container, in the order they are listed.

| | |
|---|---|
| De-ionized water | 995 ml |
| Buffer WA | 5 ml |
| Total Volume | 1,000 ml |

2. Invert gently 3–5 times.

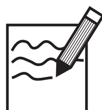
Keep Wash Solution 3 at room temperature.

*Continued on next page*

## Pre-Hybridization Procedure

Follow the procedure below to prepare your slides for hybridization.

1. Pre-heat the Pre-Hybridization Solution (prepared with Buffer WB and formamide as described on page 25) to 42°C. Place the printed slides in a polypropylene slide mailer, and fill the mailer with enough pre-heated Pre-Hybridization Solution to completely submerge the slides.
2. Close the slide mailer, and invert gently 3–5 times.
3. Incubate the slide mailer at 42°C for 30 minutes. During the incubation, fill the wash containers as described in the next step, and you can begin preparing the hybridization mixture as described on the next page.
4. Fill 5–6 wash containers (*e.g.*, Coplin jars) with de-ionized water at room temperature (15–25°C). Process each slide individually in steps 5–8. **Note:** Replace the de-ionized water in the containers after processing five slides.
5. At the end of the 42°C incubation, remove a single slide from the slide mailer. Submerge it in the first wash container and agitate for 5 seconds.
6. Transfer the slide immediately to the next wash container and agitate for 5 seconds. To avoid background spots, do not allow the liquid to dry on the slide between washes.
7. Repeat Step 6 until you have washed the slide once in each of the 5–6 wash containers.
8. To dry the slide, use one of the following methods (there should be no residue from the Pre-Hybridization Solution remaining on the dried slide):
   - Prepare a centrifuge with a microtiter plate rotor adapter that will accept the slide rack containing the array slide. (Balance the opposing arm of the rotor with a slide rack containing an equivalent number of empty slides.) Quickly transfer the slide rack with the slide to the centrifuge, and immediately spin for 2–4 minutes at 600 × *g* to dry. **Do not centrifuge at higher speeds, or the slide might break.**
   - Dry the slide under a stream of clean, filtered, compressed air. Hold the slide with the barcode at the bottom, and direct the stream of air from top to bottom, perpendicular to the slide surface. Let the water flow off smoothly and avoid streaks.
9. Repeat steps 5–8 for each slide. After all the slides are washed and dried, proceed to **Preparing the Hybridization Mixture**, next page.



## Note

- The volumes in the hybridization protocol are calculated for use with LifterSlips™ with dimensions of 25 × 60 mm. If you are using a different size of cover slip or LifterSlip™, you may adjust the volumes accordingly.
- Blocking DNA (*e.g.*, Human Cot-1 DNA®) supplied at 1 mg/ml should be concentrated to 10 mg/ml using ethanol precipitation prior to adding it directly to the hybridization reaction. See the documentation provided with the blocking agent for more information.
- In our experience, the final signal intensity depends on the total amount of labeled cDNA used for hybridization, not on the cDNA concentration. Different hybridization volumes will not significantly affect the intensity.

*Continued on next page*

**Important**

- During the following hybridization and post-hybridization procedures, be careful to minimize exposure of the labeled cDNA and array to direct light, to avoid photobleaching.
- Make sure that your hybridization incubator thermometer is calibrated.
- Always wear powder-free latex gloves when handling arrays. Avoid contact with the printed array surface.

## Preparing the Hybridization Mixture

Prepare the hybridization mixture for each slide as follows.

**Note:** For a single-color hybridization, use the entire volume of the labeling reaction (~16 µl) in the hybridization mixture. For a dual-color hybridization, use the entire volume from **both** labeling reactions (~32 µl).

1. Warm the Hybridization Solution (prepared with buffer and formamide as described on page 26) to 42°C before starting, and ensure that any precipitate is completely dissolved.
2. Prepare a hybridization mixture as follows:

| Component | Volume per slide |
|---|---|
| Hybridization Solution | 42 µl |
| Hybridization Blocking DNA, 10 mg/ml (*e.g.*, Human Cot-1 DNA®, Mouse Cot-1 DNA®, Salmon-Sperm DNA; see **Note** previous page) | 1 µl |
| Labeled cDNA (for dual-color hybridizations, combine both labeling reactions as specified above) | ~16/32 µl |
| De-ionized Water | to 84.0 µl |

3. Gently vortex and heat the hybridization mixture at 95°C for 5 minutes. Centrifuge the tube briefly to bring down any condensation.

Proceed to **Hybridization Procedure**, below.

## Hybridization Procedure

Follow the procedure below to hybridize your target DNA to the slides. Process each slide individually.

1. Place a slide prepared with Pre-Hybridization Solution (Step 9, page 27) flat with the array facing up in an open, clean, dry hybridization chamber. Place a clean, dust-free LifterSlip™ over the array area of the slide. The LifterSlip™ should be placed with the dull side of the white strips facing down along the length of the slide.
2. Position your pipette tip along an open (short) edge of the LifterSlip™, and slowly and carefully pipet 80 µl of the hot (95°C) hybridization mixture prepared as described above under the LifterSlip™ until the array surface underneath is completely covered with the mix. When pipetting, be careful not to form bubbles under the slip. If bubbles appear, try to remove them by gently tapping the LifterSlip™ with a pipette tip. Add any remaining hybridization mixture at the other corners of the LifterSlip™.

*Protocol continued on next page*

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## Hybridization Procedure, continued

*Protocol continued from previous page*

3. Repeat Steps 1–2 for each slide. After all the slides are processed, add the amount of water or buffer recommended by the manufacturer to each hybridization chamber to ensure controlled humidity. Maintaining controlled humidity during hybridization is crucial for successful microarray experiments to prevent the slides from drying out.
4. Seal the chamber and place it in a temperature-calibrated incubator at 42°C. Make sure to keep the slides flat during transfer.
5. Incubate at 42°C overnight (16–20 hours).

After incubation, proceed to **Post-Hybridization Wash 1**, below.

## Post-Hybridization Wash 1

Following hybridization and overnight incubation, proceed with the post-hybridization wash described below.

1. Preheat the Wash Solution 1 (prepared as described on page 26) to 42°C.
2. For multiple slides, place a slide rack in a wash tank and fill the tank with the preheated Wash Solution 1. Use enough wash solution to cover the entire surface of the slides.
3. Fill a squirt bottle with preheated Wash Solution 1. Use 10–15 ml per slide. Process each slide individually in Steps 4 and 5.
4. Remove a slide from the hybridization chamber (Step 5, above). Using a squirt bottle, gently spray the slide so that the LifterSlip™ falls off the slide surface and into a waste container. Thoroughly rinse both sides of the slide.

   **Note:** It is important not to scratch the surface of the slide when removing the LifterSlip™.
5. Place the slide into the slide rack in the wash tank containing pre-heated Wash Solution 1.
6. Repeat steps 4 and 5 for each slide. After all the slides are in the tank, incubate at room temperature for 10 minutes with gentle mixing (*e.g.,* on an orbital shaker).
7. Drain the tank, add fresh Wash Solution 1 at 42°C, and incubate again at room temperature for 10 minutes with gentle mixing.

After incubation, proceed with **Post-Hybridization Wash 2**, next page.

*Continued on next page*

# Microarray Hybridization, continued

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## Post-Hybridization Wash 2

1. Fill a wash tank with the Wash Solution 2 (prepared as described on page 26) at room temperature. Use enough wash solution to cover the entire surface of the slides.
2. Remove the slide rack with the slides from the Wash Solution 1 wash tank (Step 7, previous page). Submerge the rack in the wash tank containing Wash Solution 2.
3. Incubate in the wash tank at room temperature (15–25°C) for 10 minutes with gentle mixing.

After incubation, proceed with **Post-Hybridization Wash 3**, below.

---

## Post-Hybridization Wash 3

1. Fill a wash tank with Wash Solution 3 (prepared as described on page 26) at room temperature. Use enough wash solution to cover the entire surface of the slides.
2. Remove the slide rack with the slides from the Wash Solution 2 wash tank (Step 3, above), and immediately submerge it in the wash tank containing Wash Solution 3. Incubate at room temperature (15–25°C) for 1 minute.
3. Drain the tank, add fresh Wash Solution 3, and incubate again for 1 minute.
4. Drain the tank again, add fresh Wash Solution 3, and incubate again for 1 minute. Proceed immediately to Step 5. To avoid background spots, do not allow the liquid to dry on the slide.
5. To dry the slides, use one of the following methods (there should be no residue from the Wash Solution remaining on the dried slide):
    - Prepare a centrifuge with a microtiter plate rotor adapter that will accept the slide rack containing the array slide. (Balance the opposing arm of the rotor with a slide rack containing an equivalent number of empty slides.) Quickly transfer the slide rack with the slide to the centrifuge, and immediately spin for 2–4 minutes at 600 × *g* to dry. **Do not centrifuge at higher speeds, or the slide might break.**
    - Dry each slide under a stream of clean, filtered, compressed air. Hold the slide with the barcode at the bottom, and direct the stream of air from top to bottom, perpendicular to the slide surface. Let the water flow off smoothly and avoid streaks.

Scan the array within ½ hour after the final wash step, to avoid photobleaching. See **Scanning the Microarray**, next page.

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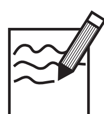
*Continued on next page*

### Scanning the Microarray

- The excitation and emission maxima of the Alexa Fluor® dyes are:

| Dye | Excitation | Emission |
|---|---|---|
| Alexa Fluor™ 555: | 555 nm | 565 nm |
| Alexa Fluor™ 647: | 650 nm | 670nm |

- The array should be shielded from direct light and scanned within ½ hour of completion the final wash, to minimize photobleaching.
- The array can be scanned using a standard digital microarray scanner. We recommend a scanner with a bit depth of at least 16 bits/pixel. The GenePix® 4000B (Molecular Devices) is a common microarray scanner, and includes software for analyzing the scanned image.
- Follow the instructions provided with your scanner for adjusting the photomultiplier tube (PMT) settings. It is important to adjust the PMT setting for each channel for maximum dynamic range and channel balance.
- Be careful to position the slide in the proper orientation in the microarray scanner. If no signal is apparent after scanning, double-check the orientation of the slide. Consult your scanner documentation for details.



**Note**

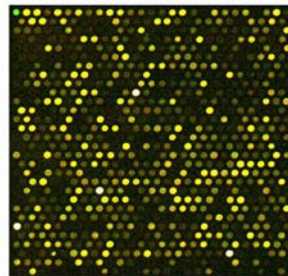
When scanning dual-color arrays, we recommend examining the image histogram (available with GenePix® Pro software) to determine whether the signal intensities in the two channels are comparable.

# Expected Results

## Example of Results

Total RNA was isolated from frozen whole mouse heart tissue (atria removed) using TRIzol® Reagent and the PureLink™ Micro-to-Midi Total RNA Purification System as described in this manual. Separate aliquots of the RNA were used to synthesize and label cDNA using the SuperScript™ Direct cDNA Labeling System with Alexa Fluor® 555-aha-dUTP and Alexa Fluor® 647-aha-dUTP, respectively. BioModule™ Microarray Hybridization Solutions were used to hybridize both labeled samples to a Corning® UltraGAPS™ slide printed with the MEEBO (Mouse Exonic Evidence-Based Oligonucleotide) oligonucleotide probe set.

The array was scanned using a GenePix® 4000B (Molecular Devices) and GenePix® Pro software. One block on the array is shown below.



# Troubleshooting

## Introduction

Review the information in this section to troubleshoot your microarray experiments.

| Problem | Possible Cause | Solution |
|---|---|---|
| **Total RNA Purification** | | |
| Low RNA yield | Incomplete lysis | • Decrease the amount of starting material used or increase the volume of TRIzol® to achieve complete lysis. The volume of tissue should not exceed 10% of the volume of TRIzol® Reagent used for homogenization.<br>• Cut tissue samples into smaller pieces and ensure the tissue is completely immersed in TRIzol® Reagent. |
| | Poor quality of starting material | • The yield and quality of RNA depends on the type and age of the starting material.<br>• Be sure to use fresh sample and process immediately after collection or freeze the sample at –80°C or in liquid nitrogen immediately after harvesting. |
| | RNA precipitated out of solution after adding ethanol to TRIzol® preparation | Add ethanol in sequential volumes as specified and mix carefully between additions. |
| | Suboptimal elution conditions | • Be sure to incubate for 1 minute before elution.<br>• To recover more RNA, perform a second elution step. |
| Low RNA yield, continued | Improper handling of sample from harvest until lysis | • If not processed immediately, quick-freeze tissue immediately after harvesting and store at –80°C or in liquid nitrogen.<br>• Frozen samples must remain frozen until TRIzol® Reagent is added.<br>• Homogenize tissues quickly after adding TRIzol® Reagent. |
| | Tissue very rich in RNases (*e.g.* pancreas) | RNA isolated from tissue rich in RNases may require the addition of RNAse inhibitors/inactivators to protect the RNA from degradation. |
| | RNA has been damaged or degraded | Re-purify RNA; follow the specified RNA handling guidelines and be careful to maintain aseptic conditions. |

*Continued on next page*

| Problem | Possible Cause | Solution |
|---|---|---|
| **Total RNA Purification, continued** | | |
| Low $A_{260}$ ratio | Sample was diluted in water; non-buffered water has variable pH (4) | Use 10 mM Tris-HCl (pH 7.5) to dilute sample for OD measurements. |
| 28S and 18S bands are not observed after agarose gel electrophoresis | Too little RNA loaded on the gel | Be sure to load at least 250 ng of RNA for analysis. |
| | RNA has been damaged or degraded | Re-purify RNA; follow the specified RNA handling guidelines and be careful to maintain aseptic conditions. |
| **cDNA Labeling** | | |
| Yield of cDNA 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.<br>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 5 µg of total RNA. |
| | Poor quality RNA used or RNA is degraded | Check the quality of your RNA preparation on a gel. 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. |
| | Concentration of NaOH and/or HCl used in the hydrolysis and neutralization reaction is incorrect. This affects the pH of the reaction and therefore may affect binding to the column. | Verify the concentration of NaOH and HCl, and repeat the reaction if necessary. |
| | cDNA has been lost in the purification step | Measure the amount of cDNA produced by the Control RNA before and after purification. Follow the purification procedure without modifications. |

*Continued on next page*

| Problem | Possible Cause | Solution |
|---|---|---|
| **cDNA Labeling, continued** | | |
| Amount of fluorescent dye/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 dyes and 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. |
| **Microarray Hybridization** | | |
| No signal or weak signal on the microarray | Hybridization or wash temperature too high | Check the temperature with a calibrated thermometer during hybridization and washing. If your array contains short oligonucleotides (<60 bases), it may be necessary to use a lower temperature for hybridization and/or washing. |
| | Hybridization time too short | Incubate the slide at 42°C for at least 16 hours. |
| | Condensation on slide surface during hybridization | Use individual hybridization chambers for each hybridization. Do not add more liquid to the chamber than recommended by the manufacturer. |
| | Photobleaching of the Alexa Fluor® dyes | Avoid direct exposure of the dyes, labeled cDNA, and hybridized array to light. Perform hybridization and wash procedures in low light conditions. |
| | Array slide scanned in wrong orientation | Check the position of the slide in the scanner; reposition and rescan if necessary. |
| Coverslip stuck to array surface | Hybridization chamber not properly sealed or humidified | Make sure that the chamber is properly sealed with the correct amount of liquid prior to incubation. |
| | Inadequate volume of Hybridization Solution used for coverslip size | Make sure that the Hybridization Solution completely covers the array surface under the LifterSlip™/coverslip. |

*Continued on next page*

# Troubleshooting, continued

| Problem | Possible Cause | Solution |
|---|---|---|
| **Microarray Hybridization, continued** | | |
| High or uneven background on the array | Residual wash solutions dried on microarray slide | Transfer the slide quickly between wash containers, and centrifuge immediately after the final wash step. Avoid exposing the slide to air between washes for more than a few seconds. Dried wash solution will appear as streaks on the slide. |
| | Dehydration of the hybridization mixture | This frequently appears as high background around the edges of the LifterSlip™/coverslip. Make sure that the hybridization mixture completely covers the array surface under the LifterSlip™/coverslip, and that humidity is maintained during incubation. |
| | Air bubbles trapped under the LifterSlip™ during hybridization | Air bubbles can prevent the target from coming into contact with the spotted probe. Make sure that LifterSlips™ are clean and dust-free before applying them to the slide. Small air bubbles may dissipate during hybridization. |
| | Flat cover slips used | Flat cover slips do not allow sufficient volume for hybridization. Use a LifterSlip™ or a cover slip with lifter bars on the sides to accommodate additional liquid volume. |
| | Scratches from LifterSlip™ | Do not try to move the LifterSlip™ after placing it on the slide. During the initial washing step after hybridization (page 29), the LifterSlip™ should glide off the slide easily. Do not attempt to remove the LifterSlip™ manually. |
| | Improper array handling | Always wear powder-free gloves when handling the array, and avoid touching the slide surface. |
| | Poor slide quality | Arrays scanned prior to hybridization should show no fluorescence. Scan a slide from each printing batch prior to hybridization. |
| | Canned air was used to dry the slide | Canned air contains propellants, which can leave a visible residue and reduce the image quality. Use a clean, filtered air source or centrifugation to dry the slides. |

*Continued on next page*

## Troubleshooting, continued

| Problem | Possible Cause | Solution |
|---|---|---|
| **Microarray Hybridization, continued** | | |
| Snowy, flocculent background on the array | Residue from pre-hybridization carried over into wash tanks | Do not use the wash tanks for pre-hybridization. Use slide mailers that are dedicated to these steps. |
| Nonspecific signals on the array | No competitor DNA in hybridization mixture | Add salmon-sperm DNA to the hybridization mixture as indicated in the procedure. |
| | Hybridization time was too long | Decrease hybridization time. Hybridize for a maximum of 20 hours. |
| | Hybridization temperature was too low | Perform hybridization at 42°C as described in the procedure. Check the temperature with a calibrated thermometer during hybridization and washing. |
| | Incomplete washing after hybridization | Make sure that the wash solutions were prepared properly (see page 26) and that all wash steps were performed as described. |

# Appendix

## Technical Service

### Web Resources

Visit the Invitrogen web site at www.invitrogen.com for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical service contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

### Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our web site (www.invitrogen.com).

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### Material Data Safety Sheets (MSDSs)

MSDSs are available on our web site at www.invitrogen.com. On the home page, click on Technical Resources and follow instructions on the page to download the MSDS for your product.

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# Product Qualification

### Introduction

Invitrogen qualifies the components of the BioModule™ Microarray Units as described below.

### TRIzol® Reagent

TRIzol® Reagent is functionally qualified by isolating RNA and DNA and qualitatively and quantitatively analyzing it in agarose gel electrophoresis. RNA is also analyzed by northern blot analysis.

### PureLink™ Micro-to-Midi Total RNA Purification System

The PureLink™ Micro-to-Midi Total RNA Purification System is functionally qualified by isolating total RNA from HeLa cells as described in this manual and must produce the following results:

- $OD_{260/280}$ between 1.9 and 2.1
- Intact RNA as determined by visual inspection on an agarose gel

In addition, each kit component is sterile and free of ribonuclease contamination, and is lot qualified for optimal performance.

### DNase I, Amplification Grade

DNase I, Amplification Grade, was analyzed for the ability to digest double-stranded DNA into oligonucleotides. DNase I and its buffers were tested for the absence of RNase activity in a digestion reaction with 0.24–9.5 Kb RNA Ladder; gel analysis showed no degradation of the RNA ladder as compared to a no-DNase I control.

### SuperScript™ Plus Indirect cDNA Labeling System

This kit was verified in replicate labeling reactions using 10 µg of total HeLa RNA, 2 µl of 2.5 µg/µl anchored oligo$(dT)_{20}$ primer, and amino-modified dNTP mix for cDNA synthesis. For the coupling step, Alexa Fluor® 555 or Alexa Fluor® 647 dyes were used.

After purification, the labeled cDNA was scanned to read the full absorbance spectrum from 240–800 nm. The amount of coupled dye was calculated using the formulas on page 22. In addition, each reaction was run on a 1.2% E-Gel to determine the quality of the product.

### SuperScript™ Plus Direct cDNA Labeling System

This kit was verified using 10 µg total HeLa or Human Placenta RNA in replicate labeling reactions with Alexa Fluor® 555-aha-dUTP and Alexa Fluor® 647-aha-dUTP. After purification, the labeled cDNA was scanned to read the full absorbance spectrum from 240–800 nm.

The amounts of incorporated nucleotides were calculated using the formulas on page 22. In addition, the length of the labeled product was determined by agarose gel electrophoresis.

# Accessory Products

## Additional BioModule™ Units

Additional BioModule™ Units that are part of the gene expression profiling system are available separately from Invitrogen. Ordering information is provided below. For more information, visit our web site at www.invitrogen.com or call Technical Service (see page 38).

| Product | Amount | Catalog no. |
|---|---|---|
| BioModule™ qRT-PCR Unit | | |
| *with Low-Throughput Purification* | 100 qPCR reactions | WFGE01 |
| *with High-Throughput Purification* | 1,000 qPCR reactions | WFGE02 |
| BioModule™ Western Analysis Unit | | |
| *for chromogenic detection* | 20 transfers | WFGE09 |
| *for chemiluminescent detection* | 20 transfers | WFGE10 |
| BioModule™ Transfection and Control Unit with BLOCK-iT™ Technology | 1 kit | WFGE06 |
| BioModule™ Immunohistochemistry (IHC) Unit | 150 slides | WFGE11 |
| BioModule™ BLOCK-iT™ Unit with Pol II miR RNAi Expression Vector | 20 reactions | WFGE07 |
| BioModule™ BLOCK-iT™ Unit with Lentiviral Pol II miR RNAi Expression System | 20 reactions | WFGE08 |

## Additional Products

Additional reagents that may be used with the BioModule™ Microarray Unit are available separately from Invitrogen. Ordering information is provided below. For more information, visit our web site at www.invitrogen.com or call Technical Service (see page 38).

| Product | Amount | Catalog no. |
|---|---|---|
| Quant-iT™ RiboGreen® RNA Assay Kit | 1 kit | R-11490 |
| RNase *AWAY*® | 250 ml | 10328-011 |
| E-Gel® 1.2% Starter Pak | 6 gels and E-Gel® PowerBase™ | G6000-01 |
| E-Gel® 1.2% 18-pak | 18 gels | G5018-01 |
| HEEBO Human Genome Set | 200 pmol<br>600 pmol | OL-10-105<br>OL-10-106 |
| MEEBO Human Genome Set | 200 pmol<br>600 pmol | OL-10-122<br>OL-10-123 |
| Human Cot-1 DNA®, 1 mg/ml | 500 µg<br>1 mg | 15279-011<br>15279-101 |
| Mouse Cot-1 DNA®, 1 mg/ml | 500 µg | 18440-016 |
| UltraPure™ Salmon-Sperm DNA Solution, 10 mg/ml | 5 × 1 ml | 15632-011 |

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