applied biosystems™
by life technologies™

Arcturus® Turbo Labeling™ Kit

Biotin, Cy®3, Cy®5 Dyes

User Guide

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14827-00 Rev. B

07/2010

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1. Introduction

1.1. ABOUT THE KIT

1.1.1. BACKGROUND

Microarrays have provided researchers with a powerful tool for gene expression profiling and have revolutionized functional genomics over the last decade. However, microarray analysis of needle biopsies, fine needle aspirates, laser capture microdissected (LCM) cells, FACS cells, or excision biopsies has been challenging because the amount of RNA obtained from such samples is usually limited. This has necessitated amplification of RNA to generate a sufficient amount of probe for hybridization.

A commonly used method of RNA amplification is the T7-polymerase-based linear amplification protocol, first described by van Gelder and Eberwine. This procedure is based on the reverse transcription of mRNA into double-stranded cDNA using an Oligo(dT) primer containing a T7 RNA polymerase promoter sequence. Linear amplification is achieved during in vitro transcription (IVT) of the double-stranded cDNA, resulting in amplified antisense RNA (aRNA).

The Turbo Labeling™ Kits enable labeling of the amplified STE, total cellular RNA or cDNA using biotin, Cy®3, or Cy®5 labeling reagents. The kits are intended for cDNA microarray, oligonucleotide array, and Affymetrix GeneChip array users.

See Figure 1.1 for a schematic overview of the Turbo Labeling process.

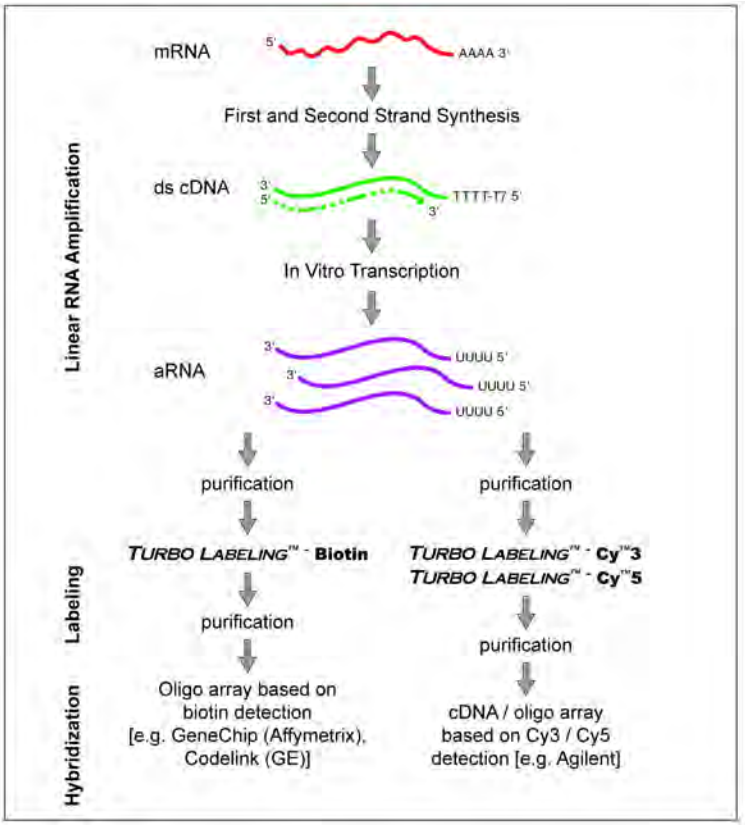

Figure 1.1: Schematic overview of the Turbo Labeling process.

1.1.2. TURBO LABELING™ KIT PRINCIPLE

This proprietary technology is based on the stable binding properties of a platinum complex to biomolecules. The molecule consists of a platinum complex, a detectable molecule, and a leaving group that is displaced upon reaction with the target. This molecule forms a coordinate bond, firmly coupling the label to the target. The label is bound to DNA or RNA by binding to the N7 position of guanine. See Figure 1.2 below.

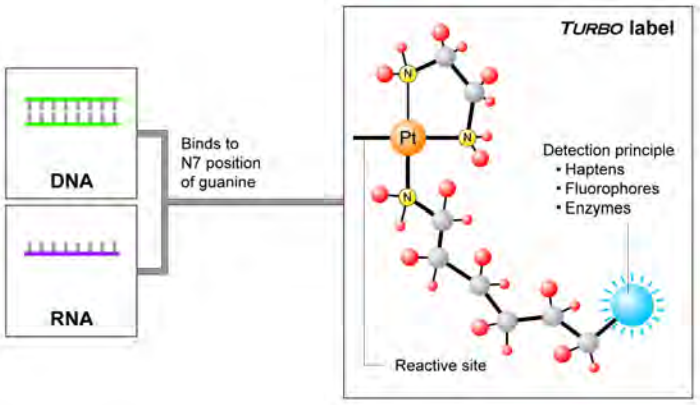

Figure 1.2: Turbo Labeling labels RNA by forming a coordinate bond on the N7 position of guanine.

1.1.3. TIME REQUIREMENTS

Turbo Labeling Kits are available with biotin, Cy3, or Cy5 labels, thereby enabling one-step, non-enzymatic labeling of nucleic acids, achievable within 30–60 minutes. Turbo Labeling can be performed with or without enzymatic amplification prior to labeling. See Figure 1.3 below.

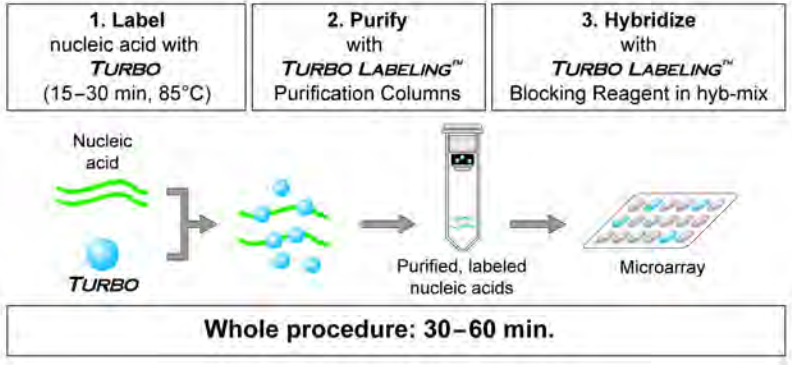

Figure 1.3: A 30- to 60-minute labeling protocol for microarray applications.

1.2. KIT COMPONENTS

Inspect and store all kit components upon receipt. For optimal results, use the reagents as soon as possible after receipt.

⚠ **IMPORTANT:** All reagents included in the kit modules should be used within 6 months of the shipment date.

1.2.1. BIOTIN REAGENTS

The Turbo Labeling-Biotin Kit (KIT0608) reagents are listed below.

Component	Volume/Quantity	Storage
Turbo Biotin Reagent	60 µl	4° to 8° C
Turbo 10X Labeling Buffer	60 µl	4° to 8° C
Turbo Blocking Reagent	1200 µl	-15° to -30° C[1]
Turbo Purification Columns	12 columns	4° to 8° C
Turbo Collection Tubes	12 tubes	4° to 8° C

1.Shipped at 4° C, stored at -15° to -30° C upon arrival.

1.2.2. Cy3 REAGENTS

The Turbo Labeling-Cy3 Kit (KIT0609) reagents are listed below.

Component	Volume/Quantity	Storage
Turbo Cy3 Reagent	60 µl	4° to 8° C
Turbo 10X Labeling Buffer	60 µl	4° to 8° C
Turbo Blocking Reagent	1200 µl	-15° to -30° C[1]
Turbo Purification Columns	12 columns	4° to 8° C
Turbo Collection Tubes	12 tubes	4° to 8° C

1.Shipped at 4° C, stored at -15° to -30° C upon arrival.

1.2.3. Cy5 REAGENTS

The Turbo Labeling-Cy5 Kit (KIT0610) reagents are listed below.

Component	Volume/Quantity	Storage
Turbo Cy5 Reagent	60 µl	4° to 8° C
Turbo 105 Labeling Buffer	60 µl	4° to 8° C
Turbo Blocking Reagent	1200 µl	-15° to -30° C[1]
Turbo Purification Columns	12 columns	4° to 8° C
Turbo Collection Tubes	12 tubes	4° to 8° C

1.Shipped at 4° C, stored at -15° to -30° C upon arrival.

1.2.4. SAFETY DATA SHEETS

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

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

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

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

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

1.3. RECOMMENDATIONS FOR NUCLEASE-FREE TECHNIQUE

1.3.1. GENERAL

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

- Use only the reagents/components that are recommended or included in the Turbo Labeling Kits. Substitutions may adversely affect performance or introduce nucleases.
- Wear disposable gloves and change them frequently to prevent the introduction of nucleases from skin surfaces. After putting on gloves, avoid touching surfaces that may introduce nucleases onto glove surfaces.
- Use nuclease-free solutions.
- Use nuclease-free glassware.
- Use only new plasticware that is certified nuclease-free.
- Use only new, sterile, nuclease-free pipette tips and microcentrifuge tubes.
- Wash scalpels, tweezers, and forceps with detergent, then rinse three times with nuclease-free water.

1.3.2. SURFACES AND EQUIPMENT

Once every day or more frequently, depending on use: Use RNase AWAY or similar decontamination reagents. Follow the manufacturer's instructions to clean any surfaces and equipment (for example, pipettors, racks, centrifuge, *etc*.) that may come in contact with samples.

2. Preparing aRNA for Turbo Labeling™ Kit

2.1. BEFORE YOU BEGIN

2.1.1. ABOUT THE TURBO LABELING™ KIT PROCESS

The procedure of expression analysis with the Turbo Labeling™ Kits is as follows:

1. aRNA is generated from isolated total RNA via linear amplification using unmodified nucleotides.
2. aRNA is cleaned to remove salts, detergents, cations, and other reagents.
3. aRNA is non-enzymatically labeled with the Turbo Labeling reagents: biotin, Cy®3, or Cy®5 reagents (15–30 minutes).
4. The labeled aRNA is purified with the Turbo Purification Columns.
5. The labeled aRNA is fragmented.
6. The labeled and fragmented aRNA is hybridized to a microarray in the presence of the Turbo Blocking Reagent.

2.1.2. MATERIALS REQUIRED

To perform the procedures in this protocol, you need the materials listed below.

Consumables	Supplier
Disposable gloves	Major Laboratory Suppliers (MLS)
RNase AWAY or other commercially available nuclease decontamination solution	Molecular BioProducts, Inc. (Cat # 10328-011)
Kimwipes or similar lint-free towels	MLS
0.5-µl Individual Flat-Cap Tubes (≥ 0.7 µl, thin-walled tubes)	MJ Research (Cat # TBI-0501, 1,000 tubes/package)
Pipette tips, nuclease-free, aerosol-resistant (assorted sizes)	MLS
Nuclease-free water	MLS
Nuclease-free, 1.5-µl tubes	MLS
Vacuum centrifuge	MLS *Note: A vacuum centrifuge is only required if the aRNA has to be concentrated.*
Desktop centrifuge	Eppendorf 5415D or similar
Microcentrifuge	MLS
Non-frost-free freezer, –65° C to –80° C	MLS
Thermal cycler	MLS
Spectrophotometer for optical density (OD) measurements	MLS
Pipettors: • 2–20 µl • 20–200 µl	MLS

2.2. TOTAL RNA EXTRACTION AND ISOLATION

Total RNA from cell cultures or tissue biopsies, frozen or Formalin-Fixed Paraffin-Embedded (FFPE), may be extracted and isolated using standard procedures. For frozen samples, the PicoPure® RNA Isolation Kit is recommended. For FFPE samples, the Paradise® RNA Extraction/Isolation Kit is recommended. Guidelines for assessing the RNA quality and quantity are detailed in the user guides for both kits:

- PicoPure RNA Isolation Kit User Guide
- Paradise Reagent System User Guide

2.3. LINEAR AMPLIFICATION

⚠ **IMPORTANT:** Linear amplification of RNA may be carried out prior to labeling with the detection moieties. RiboAmp® or RiboAmp HS RNA Amplification Kits are recommended for linear amplification of RNA from frozen tissues or cells. For linear amplification of RNA from FFPE tissues, the Paradise Reagent System is recommended. For more information, see the kit user guides:

- RiboAmp RNA Amplification Kit User Guide
- RiboAmp HS RNA Amplification Kit User Guide
- Paradise Reagent System User Guide

2.3.1. GUIDELINES

⚠ **IMPORTANT:** Enzymatic reactions should be carried out using only unmodified nucleotides. This results in better yields, longer fragments, and a stable aRNA sample.

1. Samples need to be clear of divalent cations (for example, Mg2+), salt, and other (wash) buffer components that could affect the labeling efficiency. To ensure clean aRNA for if your aRNA was generated using RiboAmp or Paradise, these steps are included and therefore do not need to be repeated:
   a. Perform two wash steps using an ethanol-based wash buffer. (The wash buffer is labeled RW in both the RiboAmp and Paradise Kits.)
   b. After the final wash, centrifuge at 16,000 x g for 2 minutes.
2. Be aware that some components in silica-based purification systems can inhibit the Turbo Labeling Kit reaction.

3. Turbo Labeling™ Procedure – Biotin Kit

3.1. PERFORMING TURBO LABELING™ – BIOTIN KIT

To perform Turbo Labeling™ with the Biotin Kit:

1 Briefly centrifuge all required reagents to collect the contents at the bottom of the tubes.

2 Pipette **1–20 µg** of aRNA into a clean, nuclease-free, 0.5 ml microcentrifuge tube. Adjust the volume to **13 µl** with nuclease-free water.

⚠ **IMPORTANT:**

a If the aRNA is too dilute, use a vacuum centrifuge to concentrate. Do not dry-down the aRNA completely. Completely drying-down aRNA causes it to solubilize inefficiently, thereby adversely affecting labeling efficiency and probe recovery.

b Ensure the final concentration in the labeling reaction is above 50 ng/µl. In general, sub-optimal labeling is achieved if the final concentration of the aRNA in the labeling mixtures is below 50 ng/µl.

3 Add the reagents:

a Add **5 µl** of Turbo Biotin Reagent.

b Add **2 µl** of Turbo 10X Labeling Buffer (final concentration of the Labeling Buffer is 1X).

Reaction Mix:

Component	Volume (µl)
aRNA (1–20 µg) + nuclease-free water	13
Turbo Biotin Reagent	5
Turbo 10X Labeling Buffer	2
Total	20

4 Mix by flicking the tube or pipetting gently up and down.

5 Using a thermocycler, incubate as follows:

- **85° C** for **30 minutes**

- **4° C** for **1–30 minutes**

⚠ **IMPORTANT:** Hold the reactions at 4° C until you are ready to use them. Do not hold for more than 30 minutes.

6 Briefly centrifuge to collect the contents at the bottom of the tube.

7 Proceed to "Removing the Free Biotin Label" below.

3.2. REMOVING THE FREE BIOTIN LABEL

To remove the free biotin label:

1 Resuspend the purification column material by vortexing.

2 Loosen the cap 1/4-turn, then snap off the bottom closure.

3 Place the column in a 2-ml collection tube (provided with the kit).

4 Centrifuge the column for **1 minute** at **16,000 x g**.

Note: The rpm for a given centrifugal force (g) vary depending on the centrifuge model you are using. Be sure you select the appropriate speed to achieve the appropriate centrifugal force.

5 Discard the flowthrough and retain the collection tube.

6 To wash the column:

a Pipette **300 µl** of nuclease-free water onto the column.

b Centrifuge the column for **1 minute** at **16,000 x g**.

Note: The flowthrough volume will be 300 µl.

7 Discard the flowthrough and the collection tube.

8 Transfer the column to a new, nuclease-free, 1.5-ml microcentrifuge tube (not provided with the kit).

9 Pipette the biotin-labeled aRNA onto the column bed.

10 Centrifuge the column for **1 minute** at **16,000 x g**.

11 Discard the column and retain the flowthrough, which is the purified, labeled aRNA.

Note: The flowthrough volume will be 20 µl.

12 Quantify the amount of biotin-labeled aRNA recovered by measuring absorbance at 260 nm.

3.3. PREPARING THE LABELED MATERIAL FOR HYBRIDIZATION

Follow the hybridization procedure recommended by the array manufacturer, with the following modification:

Add the Turbo Blocking Reagent at 1 part of Blocking Reagent to 4 parts of hybridization mix (for example, 20 µl of Turbo Blocking Reagent in a total hybridization volume of 100 µl).

Note:

1. *The Turbo Blocking Reagent is used to reduce the fluorescence background on arrays.*
2. *The total by volume of hybridization mix does not change.*
3. *The addition of Blocking Reagent is compensated by lowering the amount of nuclease-free water added to the hybridization mix.*

See Appendix B for an example of the protocol for hybridization on to a GeneChip® Array (Affymetrix).

4. Turbo Labeling™ Procedure – Cy3 and Cy5 Kits

4.1. PERFORMING TURBO LABELING™ – Cy3 AND Cy5 KITS

To perform Turbo Labeling™ with the Cy®3 or Cy®5 Kits:

1 Briefly centrifuge all required reagents to collect the contents at the bottom of the tubes.

2 Pipette **1–15 µg** of aRNA into a clean, nuclease-free, 0.5-ml microcentrifuge tube. Adjust the volume to **40 µl** with nuclease-free water.

⚠ IMPORTANT:

a If the aRNA is too dilute, use a vacuum centrifuge to concentrate. Do not dry-down the aRNA completely. Completely drying-down aRNA causes it to solubilize inefficiently, thereby adversely affecting labeling efficiency and probe recovery.

b Ensure the final concentration in the labeling reaction is above 50 ng/µl. In general, sub-optimal labeling is achieved if the final concentration of the aRNA in the labeling mixtures is below 50 ng/µl.

3 Add the reagents:

a Add **5 µl** of Turbo Cy3 or Cy5 Reagent.

b Add **5 µl** of Turbo 10X Labeling Buffer (final concentration of the Labeling Buffer is 1X).

Reaction Mix:

Component	Volume (µl)
aRNA (1–15 µg) + nuclease-free water	40
Turbo Cy3 or Cy5 Reagent	5
Turbo 10X Labeling Buffer	5
Total	50

4 Mix by flicking the tube or pipetting gently up and down.

5 Using a thermocycler, incubate as follows:

- **85° C** for **15 minutes**

- **4° C** for **1–30 minutes**

⚠ **IMPORTANT:** Hold the reactions at 4° C until you are ready to use them. Do not hold for more than 30 minutes.

6 Briefly centrifuge to collect the contents at the bottom of the tube.

7 Proceed to "Removing the Free Cy3 or Cy5 Label" below.

4.2. REMOVING THE FREE Cy3 OR Cy5 LABEL

To remove the free Cy3 or Cy5 label:

1 Resuspend the purification column material by vortexing.

2 Loosen the cap 1/4-turn, then snap off the bottom closure.

3 Place the column in a 2-ml collection tube (provided with the kit).

4 Centrifuge the column for **1 minute** at **16,000 x g**.

Note: The rpm for a given centrifugal force (g) vary depending on the centrifuge model you are using. Be sure you select the appropriate speed to achieve the appropriate centrifugal force.

5 Discard the flowthrough and retain the collection tube.

6 To wash the column:

a Pipette **300 µl** of nuclease-free water onto the column.

b Centrifuge the column for **1 minute** at **16,000 x g**.

Note: The flowthrough volume will be 300 µl.

7 Discard the flowthrough and the collection tube.

8 Transfer the column to a new, nuclease-free, 1.5-ml microcentrifuge tube (not provided with the kit).

9 Pipette the Cy3- or Cy5-labeled aRNA onto the column bed.

10 Centrifuge the column for **1 minute** at **16,000 x g**.

11 Discard the column and retain the flowthrough, which is the purified, labeled aRNA.

Note: The flowthrough will be 50 µl.

12 Quantify the amount of Cy3- and Cy5-labeled aRNA and calculate Frequency of Incorporation (FOI) as mentioned in Appendix B.

4.3. PREPARING THE LABELED MATERIAL FOR ARRAY HYBRIDIZATION

4.3.1. ARRAY HYBRIDIZATION FOR AGILENT ARRAYS

Perform array hybridizations following manufacturer's recommendations.

4.3.2. ARRAY HYBRIDIZATION FOR OTHER OLIGONUCLEOTIDE ARRAYS

Follow the hybridization procedure recommended by the array manufacturer, with the following modification:

Add the Turbo Blocking Reagent at 1 part of Blocking Reagent to 3 parts of hybridization mix (for example, 25 µl of Turbo Blocking Reagent in a total hybridization volume of 100 µl).

Note:

1. *This is different from the Biotin Protocol. The Turbo Blocking Reagent is used to reduce the fluorescence background on arrays.*
2. *The total volume of hybridization mix does not change.*
3. *The addition of the Blocking Reagent is compensated by lowering the amount of nuclease-free water added to the hybridization mix.*

A. Appendix: Troubleshooting

A.1. PREPARING aRNA FOR TURBO LABELING™ KIT

Observation	Possible Cause	Recommended Action
Frequency of incorporation too low.	Salts and other impurities may affect the efficiency of labeling.	Clean up the aRNA using cleanup kits that use silica-based columns such as PicoPure. See Section 2.3.1., *"Guidelines"* on page 13.
	Incorrect ratio of labeling reagent to aRNA.	• Be sure you are using the recommended amount of Turbo Labeling™ reagents per µg of aRNA. • Be sure the concentration of the labeling reaction is above 50 ng/µl.
High labeling density.	Incorrect ratio of labeling reagent to aRNA.	Be sure you are using the recommended amount of Turbo label per µg of aRNA.

A.2. ARRAY HYBRIDIZATION AND DETECTION

Observation	Possible Cause	Recommended Action
Background on the array.	Too much probe loaded onto the microarray.	Reduce the sample amount.
	Insufficient blocking.	Add the Turbo Blocking Reagent when performing hybridization.
	Incompatibility between Turbo Blocking Reagent and other components in hybridization cocktail.	Contact Technical Support Toll-free (US): (800) 831-6844 option 5.

B. Appendix: Related Protocols

B.1. HYBRIDIZATION OF BIOTIN-LABELED aRNA ON GENECHIP® ARRAYS

B.1.1. aRNA FRAGMENTATION

1 Label 0.5 ml tubes with a corresponding sample names.

2 Mix Probe Solution as indicated in the highlighted area of Table B.1.

a Determine the volume of Biotin-aRNA to be added to the mix:

$$\text{Volume of biotin-aRNA (µl)} = \frac{\text{The desired mass of aRNA (µg)}}{\text{aRNA concentration (µg/µl)}}$$

b The total volume of the fragmentation mix should be 25 µl.

Table B.1: Fragmentation Mix

	Volume Needed (µl)	**Total Volume (µl)**
Biotin-aRNA (15mg)		
Nuclease Free Water	**Adjust to 20**	20
5x fragmentation buffer[1]	**5**	25

1.Refer to Appendix B.2., *"Preparing 5X Fragmentation Buffer"*.

3 Mix thoroughly by flicking, and spin down briefly.

Note: Do not vortex.

4 Incubate at **94° C** for **10 minutes** to Fragment RNA.

Note: Do not exceed 10 minutes.

5 Immediately incubate the samples at **4° C** for **2 minutes**, and then spin down.

6 As the samples are incubating, prepare the hybridization mix as indicated in the highlighted area of Table B.2.

Table B.2: Hybridization Mix

	Volume Needed (µl)	Total Volume (µl)
B2 Oligo	**5**	5
20X Hyb controls	**15**	20
Herring sperm DNA	**3**	23
BSA	**3**	26
2X Hyb Buffer	**150**	176
DMSO	**30**	206
Turbo Blocking Reagent	**60**	266
Water	**9**	275

7 Add **275 µl** of hybridization mix to **25 µl** of the fragmented aRNA. Maintain the mixtures at 4° C at this point.

8 Incubate the samples at **99° C** for **5 minutes**, **45° C** for **5 minutes** and then leave it at room temperature until hybridization on to arrays.

Note: Hybridize the assays within one hour of probe preparation.

B.1.2. ARRAY HYBRIDIZATION

1 Prepare the arrays for hybridization following manufacturer's recommendations.

2 Load 200 µl of probe to GeneChip arrays following manufacturer's instructions.

B.2. PREPARING 5X FRAGMENTATION BUFFER

Composition of 5X Fragmentation Buffer:

- 200 mM Tris-acetate, pH 8.2
- 500 mM KOAc
- 150 mM MgOAc

⚠ **IMPORTANT:** Prepare the 5X Fragmentation Buffer with nuclease-free reagents.

Note: Tris-containing solutions should not be treated with DEPC; however, once water has been DEPC-treated and autoclaved, it can be used for making the Tris solution.

To prepare the 5X Fragmentation Buffer:

1 Combine the following components for a total volume of 20 ml:

Component	Volume
1 M Tris acetate, pH 8.1 (Trizma Base, pH adjusted with glacial acetic acid)	4.0 ml
MgOAc	0.64 g
KOAc	0.98 g
Nuclease-free water	To bring volume to 20 ml
Total	20 ml

Note: The final pH, without adjustment, should be 8.2.

2 Mix thoroughly by vortexing.

3 Filter through a 0.2-mm vacuum filter unit.

4 Aliquot and store at room temperature.

B.3. DETERMINING THE FREQUENCY OF INCORPORATION (FOI)

The procedure below can be used to determine if the FOI of the Cy®3 or Cy®5 fluorescent dyes is sufficient for microarray hybridization.

To determine the FOI:

1. Measure the absorbance of the Cy3- or Cy5-labeled probe:
   - For Cy3 dye, absorbance is read at 260 and 550 nm.
   - For Cy5 dye, absorbance is read at 260 and 650 nm.
2. Calculate the FOI of Cy3 dye:

   $(OD_{550}/0.15) \times (324)/(OD_{260} \times 40)$
3. Calculate the FOI of Cy5 dye:

   $(OD_{650}/0.25) \times (324)/(OD_{260} \times 40)$
4. Determine the status:

If the FOI reading is...	Then...
>20	The probe is adequately labeled and is suitable for hybridization.
Between 15 and 20	The FOI reading is lower than recommended, but the probe is still usable. *Note: Signal issues with the final array data might be due to the lower FOI.*
<15	The labeled probe should be treated with caution; it may not be suitable for hybridization to microarrays.

C. Appendix: Reagent Kits

C.1. FORMALIN-FIXED PARAFFIN-EMBEDDED (FFPE)

Cat #	Description	Quantity
KIT0305	Paradise® Whole Transcript RT Reagent System	12 samples
KIT0315	Paradise Whole Transcript RT Reagent System	48 samples
KIT0312	Paradise Staining, Extraction Isolation and Amplification System	12 samples

C.2. RNA LINEAR AMPLIFICATION

Cat #	Description	Quantity
KIT0521	RiboAmp® RNA Amplification Kit (12 × 1 or 6× 2-round samples)	6–12 samples
KIT0525	RiboAmp HS RNA Amplification Kit (6 × 2-round samples)	6 × 2-round samples

C.3. SAMPLE PREPARATION

Cat #	Description	Quantity
KIT0103	PicoPure® DNA Extraction Kit	150/30 ext
KIT0204	PicoPure RNA Extraction and Isolation Kit	40 isolations

C.4. STAINING

Cat #	Description	Quantity
KIT0401	HistoGene® LCM Frozen Section Staining Kit: Includes dehydration chemicals, stain, slides and slide jars for processing 72 slides.	72 slides
KIT0420	HistoGene LCM Immunofluorescence Staining Kit: Includes staining components only.	32 slides

D. Appendix: Quick Reference Guide

D.1. TURBO LABELING KIT – BIOTIN (KIT0608)

D.1.1. BIOTIN LABELING PROCEDURE

1 Starting with **1-20 µg** of aRNA in **13 µl** of nuclease-free water, add **5 µl** Turbo Biotin reagent and **2 µl** Turbo 10x labeling buffer:

Component	Volume (µl)
aRNA (1-20 ug) + water	13
Turbo Biotin Reagent	5
10x Labeling Buffer	2
Total	20

2 Mix by flicking or pipetting gently and incubate on a thermocycler:

- **85° C** for **30 minutes**
- **4° C** for **1-30 minutes**

3 Briefly centrifuge to collect tube contents.

D.1.2. PURIFICATION PROCEDURE

1 Briefly vortex purification column, loosen cap 1/4 turn and snap off bottom closure.

2 Place tube in provided 2 ml collection tube.

3 Centrifuge column at **16,000** x g for **1 minute**.

4 Discard flow-through and retain collection tube.

5 Wash column by pipetting **300 µl** of nuclease-free water onto the column and centrifuge at **16,000 x g** for **1 minute**.

6 Discard flow-through and collection tube.

7 Transfer column to a new 1.5 ml micro-centrifuge collection tube.

8 Pipette biotin-labeled aRNA onto column bed.

9 Centrifuge at **16,000 x g** for **1 minute**.

10 Discard column and retain flow-through which is purified labeled aRNA (**20 µl**).

11 Quantify the amount of biotin-labeled aRNA recovered and proceed to hybridization as detailed in Appendix B.

D.2. TURBO LABELING KIT – CY3 (KIT0609) /CY5 (KIT0610)

D.2.1. Cy3/Cy5 LABELING PROCEDURE

1 Starting with **1-15 µg** of aRNA in **40 µl** of nuclease-free water, add **5 µl** Turbo Cy3 or Cy5 reagent and **5 µl** Turbo 10x labeling buffer:

Component	Volume (µl)
aRNA (1-15 ug) + water	40
Turbo Cy3 or Cy5 Reagent	5
10x Labeling Buffer	5
Total	50

2 Mix by flicking or pipetting gently and incubate in thermocycler:

- **85° C** for **15 minutes**
- **4° C** for **1-30 minutes**

3 Briefly centrifuge to collect tube contents.

D.2.2. PURIFICATION PROCEDURE

1 Briefly vortex purification column, loosen cap 1/4 turn and snap off bottom closure.

2 Place tube in provided 2 ml collection tube.

3 Centrifuge column at **16,000 x g** for **1 minute**.

4 Discard flow-through and retain collection tube.

5 Wash column by pipetting **300 µl** of nuclease-free water onto the column and centrifuge at **16,000 x g** for **1 minute**.

6 Discard flow-through and collection tube.

7 Transfer column to a new 1.5 ml micro-centrifuge collection tube.

8 Pipette Cy3- or Cy5-labeled aRNA onto column bed.

9 Centrifuge at **16,000 x g** for **1 minute**.

10 Discard column and retain flow-through which is purified labeled aRNA (**50 µl**).

11 Determine the FOI and proceed to hybridization as detailed in Appendix B.

Part Number 14827-00 Rev. B 07/2010

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