Label IT® Nucleic Acid Labeling Kits

Protocol for MIR 3100, 3125, 3200, 3225, 3300, 3325, 3400, 3425, 3600, 3625, 3700, 3725, 3800, 3825, 4100, 4125, 7100, 7125

Quick Reference Protocol, SDS and Certificate of Analysis available at mirusbio.com/3100

INTRODUCTION

The *Label* IT® Nucleic Acid Labeling Technology is designed to efficiently and reproducibly attach marker molecules to any nucleic acid sample in a one-step, scalable reaction. Unlike other non-radioactive labeling technologies (e.g. random priming, nick translation), the *Label* IT® Nucleic Acid Labeling Reagent covalently attaches CX-rhodamine, fluorescein, MFP488, digoxin, biotin, Cy®3, Cy®5, dinitrophenol (DNP) or TM-rhodamine to bases within DNA or RNA without dramatically altering the starting nucleic acid or hindering downstream hybridization performance. Because *Label* IT® reactions are non-destructive to the nucleic acid starting material, the *Label* IT® technology can be used in a wide variety of *in vivo*, *in vitro* and molecular biology applications.

The standard labeling procedure outlined in this protocol will yield an approximate labeling density of one *Label* IT® label per every 20-60 base pairs. This labeling density is sufficient to allow sensitive detection for most applications, though lower densities may be required for certain applications in which the labeled nucleic acids will be used for gene expression or RNAi studies. If an alternative labeling density is required, simply increase or decrease the amount of labeling reagent in the reaction or adjust the reaction incubation time.

SPECIFICATIONS

Storage	Store *Label* IT® Reagent at -20°C in both dried and reconstituted forms. Store Reconstitution Solution, 10X Labeling Buffer A, Denaturation Reagent D1 and Neutralization Buffer N1 at -20°C. Store G50 microspin columns at 4°C. DO NOT FREEZE.
Product Guarantee	The *Label* IT® Reagent is stable at -20°C for 6 months after reconstitution. Unreconstituted *Label* IT® Reagent and all other reagents are guaranteed 1 year from the date of purchase, when properly stored and handled.
Kit Size	Each Full Size Kit contains sufficient reagents to label 100 µg nucleic acid. Each Trial Size Kit contains sufficient reagents to label 25 µg nucleic acid.

Cap the *Label* IT® Reagent tightly and avoid exposure to moisture and light.

For Research Use Only.

MATERIALS

Materials Supplied

Label IT® Nucleic Acid Labeling Kits are supplied in ***one*** of the following formats:

Label IT® Product Name	Excitation Wavelength (nm)	Emission Wavelength (nm)	Quantity	Product No.
Label IT® CX-Rhodamine Labeling Kit	576	597	Full Size	MIR 3100
			Trial Size	MIR 3125
Label IT® Fluorescein Labeling Kit	492	518	Full Size	MIR 3200
			Trial Size	MIR 3225
Label IT® Digoxin Labeling Kit	n/a	n/a	Full Size	MIR 3300
			Trial Size	MIR 3325
Label IT® Biotin Labeling Kit	n/a	n/a	Full Size	MIR 3400
			Trial Size	MIR 3425
Label IT® Cy™3 Labeling Kit	550	570	Full Size	MIR 3600
			Trial Size	MIR 3625
Label IT® Cy™5 Labeling Kit	649	670	Full Size	MIR 3700
			Trial Size	MIR 3725
Label IT® DNP Labeling Kit	n/a	n/a	Full Size	MIR 3800
			Trial Size	MIR 3825
Label IT® TM-Rhodamine Labeling Kit	546	576	Full Size	MIR 4100
			Trial Size	MIR 4125
Label IT® MFP488 Labeling Kit	501	523	Full Size	MIR 7100
			Trial Size	MIR 7125

The following components are included in the *Label* IT® Nucleic Acid Labeling Kits:

Kit Component	Full Size	Trial Size	Reagent Cap Color
Label IT® Reagent	dried pellet	dried pellet	varies with reagent
Reconstitution Solution	100 µl	100 µl	brown
10X Labeling Buffer A	500 µl	100 µl	orange
Denaturation Reagent D1	500 µl	150 µl	blue
Neutralization Buffer N1	500 µl	200 µl	white
G50 microspin purification columns	20	5	N/A

Materials required, but not supplied

- Molecular biology-grade water
- Microcentrifuge tubes
- Nucleic acid sample (starting material)
- Microcentrifuge
- Optional: Materials for EtOH purification
- Optional: Detection reagents

BEFORE YOU START:

Important Tips for Optimal Nucleic Acid Labeling

The suggestions below generally yield strong labeling with minimal background and will maximize performance with most applications.

- **Reagent preparation.** Prior to first use, warm the vial containing the *Label* IT® Reagent to room temperature and centrifuge briefly (pulse) to collect the dried pellet. For subsequent uses, warm the vial of reconstituted *Label* IT® Reagent to room temperature before opening.
- **Reaction scalability.** The *Label* IT® labeling reactions can be scaled up or down to label different amounts of sample as required for alternate reaction conditions. When adjusting reactions volumes, maintain a 1X final concentration of Labeling Buffer A and ensure that the *Label* IT® Reagent does NOT constitute greater than 20% of total reaction volume.
- **Labeling ratio.** The 1:1 (v:w) ratio of *Label* IT® Reagent to nucleic acid outlined in this protocol typically results in a labeling density of 1 label per every 20-60 bases of nucleic acid. This density is suitable for most applications. However, lower labeling densities (e.g. 0.1:1 – 0.5:1) are recommended for applications for which the labeled DNA will be used for gene expression studies *in vivo*. To modify the labeling density of the sample, simply increase or decrease the amount of *Label* IT® Reagent used in the reaction or adjust the reaction incubation time, as the labeling reaction is linear over the first three hours of incubation at 37°C.
- **DNase and RNase-free materials.** Wear gloves at all times when working with RNA. Use DNase-free and RNase-free reagents and plasticware.
- **Addition of *Label* IT® Reagent.** Add the *Label* IT® Reagent to the labeling reaction last.
- **Post-labeling purification.** It is generally acceptable to assume 100% recovery of the labeled nucleic acid following G50 microspin column purification. However, if the labeled sample will be quantified by spectrophotometry, purification by ethanol precipitation is recommended as G50 microspon column purification leads to erroneously high ultraviolet A260 readings.
- **Determining the Labeling Density of the Nucleic Acid Sample.** A labeling density of 1 label per every 20-60 bases of nucleic acid can be expected if using a 1:1 (v:w) ratio of *Label* IT® Reagent to nucleic acid. If it is necessary to determine the exact labeling density of your sample, see instructions on our website in *Label* IT® Frequently Asked Questions or Tips from the Bench. The relative density of labels on purified, labeled nucleic acid can be estimated by one of the following methods:

  For fluorescent dyes:

  1. Spectrophotometric absorbance at λ_{max} of the dye. Several micrograms of sample may be required to generate significant λ_{max} absorbance readings.
  2. Fluorescent microscopy. Spot serial dilutions of purified labeled sample onto a glass slide and view with a fluorescent microscope

  For non-fluorescent dyes:

  1. Dot blot analysis. Fix dilutions of the labeled sample to a membrane, then detect with appropriate reagents.
  2. Gel shift analysis. A labeled sample may demonstrate a distinct reduction in electrophoretic mobility compared to unlabeled control sample.

A 1:1 (v:w) *Label* IT® Reagent to nucleic acid labeling ratio results in a labeling density suitable for most applications. Lower labeling densities may be required for some applications.

LABELING PROTOCOL

The procedure below describes how to perform a standard nucleic acid labeling reaction.

A. Prepare *Label* IT® Nucleic Acid Labeling Reagent

1. Before the first use, warm the *Label* IT® Reagent vial to room temperature and centrifuge briefly (pulse) to collect the dried pellet.
2. Warm the Reconstitution Solution to room temperature. The Reconstitution Solution remains frozen at 4° C. Please ensure that it is completely thawed before use.
3. For the first use only, add the pre-warmed Reconstitution Solution to each *Label* IT® pellet according to the following table:

Product MIR No.	Reconstitution Solution Volume
3125, 3225, 3325, 3425, 3625, 3725, 3825, 4125, 7125	25 µl to each *Label* IT® pellet
3100, 3200, 3300, 3400, 3600, 3700, 3800, 4100, 7100	100 µl to each *Label* IT® pellet

4. To ensure complete reconstitution of the pellet, mix well by vortexing and centrifuge briefly (pulse) to collect the solution.

B. Label Nucleic Acid Sample

1. Prepare the labeling reaction according to the example shown below. Add the reagents in the order listed, and be sure to add the *Label* IT® Reagent last.

Standard Nucleic Acid Labeling Reaction	
DNase-, RNase-free (molecular biology-grade) water	35 µl
10X Labeling Buffer A	5 µl
1 mg/ml nucleic acid sample	5 µl
Label IT® Reagent	5 µl
Total Volume	**50 µl**

Increase or decrease the amount of *Label* IT® Reagent in the reaction or adjust the reaction incubation time to modify the labeling density. The *Label* IT® Reagent should not exceed 20% of the total reaction volume.

NOTE: This example labels 5 µg of nucleic acid at a 1:1 (v:w) ratio of *Label* IT® Reagent to nucleic acid. This ratio results in labeling efficiencies that are appropriate for most applications. Increase or decrease the amount of *Label* IT® Reagent in the reaction or adjust the reaction incubation time to modify the labeling density.

C. Incubate the Reaction at 37°C for 1 hour.

NOTE: After 30 minutes of incubation, briefly centrifuge the reaction to minimize the effects of evaporation and maintain the appropriate concentration of the reaction components.

D. Purify Samples Using G50 Microspin Purification Columns (Standard Method)

NOTE: If the labeled sample will be quantified by spectrophotometry, purification by ethanol precipitation is recommended as G50 microspin column purification can lead to erroneously high ultraviolet A_{260} readings (see **Section E for recommended Ethanol Precipitation Protocol**). It is generally acceptable to assume 100% recovery of the labeled nucleic acid following microspin column purification.

1. To prepare the G50 microspin column for use, vortex briefly to resuspend the resin in the column.

2. Loosen the cap on the column by one-quarter turn and pull out the bottom closure.
3. Place the column in a sterile 1.5 ml microcentrifuge tube for support.
4. Centrifuge the column for 1 minute at 735 x g, and discard the buffer collected during the spin.
5. Place the column in a new 1.5 ml microcentrifuge tube.
6. Slowly apply the 50 µl sample to the top center of the resin without disturbing the resin bed. NOTE: The volume applied to the column must be 50 µl. If the reaction volume is lower, bring the volume to 50 µl with 1X Labeling Buffer A. If the volume exceeds 50 µl, split the reaction and use 50 µl per column.
7. Centrifuge the column at 735 x g for 2 minutes. The purified sample will collect in the microcentrifuge tube.
8. Discard the column and cap the support tube. The labeled sample is now ready for use.
9. Store the *Label* IT® labeled nucleic acid on ice for immediate use or at -20°C for long-term storage, protected from light.

E. Purification using Ethanol Precipitation (Alternative Method)

NOTE: This method is provided as an alternative to G50 microspin column purification and is optimal if quantification of the labeled nucleic acid is necessary. For labeling reaction volumes <100 µl, bring the volume up to 100 µl with 1X Labeling Buffer A or molecular biology-grade water before adding sodium chloride and ethanol.

Ethanol purification of the *Label* IT® labeled nucleic acid is optimal if spectrophotometric quantification is required.

1. Add 0.1 volume of 5M sodium chloride and 2 - 2.5 volumes of ice cold 100% ethanol to the reaction. Mix well and place at ≤ -20°C for at least 30 minutes.
2. Centrifuge at full speed (>14,000 x g) in a refrigerated microcentrifuge for 15-30 minutes to pellet the labeled nucleic acid. Once pelleted, gently remove the ethanol with a micropipetter; do not disturb the pellet. NOTE: Small nucleic acid quantities can be difficult to visualize. Mark and orient the precipitate-containing tubes in the microfuge such that the pellet will form in a predetermined place.
3. Wash the pellet once with 500 µl room temperature 70% ethanol. Centrifuge at full speed for an additional 15-30 minutes.
4. Remove all traces of ethanol with a micropipetter. DO NOT allow the sample to dry longer than 5 minutes as the pellet may become difficult to resuspend.
5. Resuspend the *Label* IT® labeled nucleic acid in an appropriate volume of 1X Labeling Buffer A or sterile water.
6. If an exact nucleic acid concentration is required, quantify the purified, labeled nucleic acid on a spectrophotometer and dilute to the desired working concentration.
7. Store the purified, labeled nucleic acid on ice for immediate use or at -20°C for long-term storage. Protect the *Label* IT® labeled sample from light.

APPLICATION NOTES

A. *In Vitro* Tracking Experiments

Subcellular localization and target gene functionality can be monitored in the same experiment following the delivery of the labeled sample into mammalian cells in culture. The *Label* IT® Tracker™ and *Label* IT® siRNA Tracker Intracellular Localization Kits are specifically tailored for effective and nondestructive labeling of plasmid DNA or siRNA for *in vitro* nucleic acid tracking applications. To identify the ideal *Trans*IT® transfection reagent for labeled DNA/siRNA delivery to your cell type, see the Related Products Section (page 8) or visit the Reagent Agent Transfection Database at www.mirusbio.com/ra.

B. *In Vivo* Tracking Experiments

Subcellular localization and reporter transgene expression can be monitored following the introduction of labeled nucleic acid into mammalian cells *in vivo*. The *Trans*IT®-EE and *Trans*IT®-QR Hydrodynamic Delivery Solutions are designed specifically for the safe and efficient delivery of nucleic acids into laboratory mice using the hydrodynamic tail vein injection procedure. Nucleic acids delivered with these kits primarily target the liver, with lower levels of expression detected in the spleen, lung, heart and kidneys.

Use RNase and DNase-free components.

C. Hybridization Reaction Using Labeled DNA Samples

For optimal sensitivity and stability of labeled DNA samples in hybridization reactions, use the supplied Denaturation Reagent D1 and Neutralization Buffer N1. NOTE: Do not heat-denature the labeled DNA probe prior to D1 and N1 treatment. Once treated with Denaturation Reagent D1 and Neutralization Buffer N1, labeled samples can be heat denatured as required for hybridization applications. The following procedure is recommended:

1. Immediately prior to the hybridization, add 0.1 volume of Denaturation Reagent D1 to the labeled sample. Mix well and incubate for 5 minutes at room temperature.
2. Add 0.1 volume of Neutralization Buffer N1. Mix well and incubate on ice for a minimum of 5 minutes. The labeled sample is now ready to use in any hybridization protocol. If the denatured sample will be used at a later time, store at -20°C and avoid multiple freeze/thaws to maintain the denatured state.

D. Hybridization Reactions Using Labeled RNA Samples

For optimal sensitivity and stability of the *Label* IT® labeled RNA probe, denature the RNA by heating at 55-65°C for 10 minutes prior to any hybridization applications. Do not denature the labeled RNA probe with Denaturation Reagent D1 and Neutralization Buffer N1, as alkaline conditions can hydrolyze RNA.

E. Biotin Detection

Tracking Biotin-labeled nucleic acids allows the use of a wide variety of commercially available detection reagents for *in vivo* or *in vitro* applications. The potential for multi-color tracking experiments is enhanced when the experimental design includes detection of a Biotin-labeled plasmid with a unique fluorophore conjugate and the direct detection of Cy®3, Cy®5, Fluorescein, MFP488, TM-Rhodamine or CX-Rhodamine-labeled plasmid(s). For a post-labeling avidin/streptavidin conjugation procedure for cells grown on coverslips, see the *Label*IT® Tracker Intracellular Localization Kit protocol.

TROUBLESHOOTING GUIDE

Problem	Solution
Suboptimal Nucleic Acid Labeling	
Poor quality of nucleic acid sample	Use purified nucleic acid (A_{260}/A_{280} between 1.8 and 2.2) that is free from proteins, carbohydrates, etc.
	Avoid nucleic acid degradation by using DNase- and RNase-free handling procedures and plasticware.
Incomplete labeling reaction	Incubate the reaction at 37°C for 1 hour. The reaction may be extended to 2 hours to increase the labeling density.
	A quick spin after 30 minutes will minimize the effect of evaporation.
Insufficient volume of *Label* IT® Reagent added to the reaction	Use 1 µl of *Label* IT® Reagent per 1 µg of nucleic acid. See 'Labeling Protocol' (page 4) for proper labeling reaction setup.
Labeling reaction was not scaled properly	Keep the volume of *Label* IT® Reagent less than 20% of the total reaction volume, and ensure that the final concentration of Labeling Buffer A is 1X.
	Avoid using nucleic acid samples in high salt, as NaCl concentrations greater than 50 mM can inhibit the labeling reaction.
Improper storage of reagents	Store both reconstituted and unreconstituted *Label* IT® Reagent tightly capped at -20°C, and protect from exposure to light and moisture.
	Warm vial to room temperature before opening.
Microspin columns were not stored properly	Store columns at 4°C. DO NOT FREEZE. Do not use columns if they have been frozen.
Nucleic acid pellets were allowed to over-dry (after EtOH Purification)	Do not allow the labeled nucleic acid pellet to dry extensively after ethanol precipitation. Remove all traces of the ethanol wash and resuspend immediately in 1X Buffer A or a low salt buffer of choice.
Incorrect use of the Denaturation Reagent D1 and Neutralization Buffer N1	Labeled DNA samples intended for hybridization applications must be treated with Denaturation Reagent D1 and Neutralization Buffer N1 as described in 'Application Notes' (page 6). This procedure denatures the DNA and stabilizes the *Label* IT® Labels. Labeled DNA samples treated with Denaturation Reagent D1 and Neutralization Buffer N1 can be heat denatured if required. Do not heat denature labeled DNA before treating with Denaturation Reagent D1 and Neutralization Buffer N1.
	Do not denature labeled RNA with Denaturation Reagent D1 and Neutralization Buffer N1, as alkaline conditions can destroy RNA. For optimal sensitivity and stability of the *Label* IT® labeled RNA probe, denature the RNA by heating at 55-65°C for 10 minutes prior to any hybridization applications.

RELATED PRODUCTS

- *Label* IT® Tracker™ Intracellular Localization Kits
- *Label* IT® Tracker siRNA Intracellular Localization Kits
- *Label* IT® Plasmid Delivery Controls
- *Label* IT® RNAi Delivery Controls
- Ingenio® Electroporation Solution and Kits
- *Trans*IT-X2® Dynamic Delivery System
- *Trans*IT®-2020 Transfection Reagent
- *Trans*IT®-LT1 Transfection Reagent
- *Trans*IT® Cell Line Specific Transfection Reagents and Kits
- *Trans*IT®- EE Hydrodynamic Delivery Solution and Starter Kit
- *Trans*IT®-QR Hydrodynamic Delivery Solution and Starter Kit

For details on our products, visit www.mirusbio.com.

Contact Mirus Bio for additional information.

Mirus Bio LLC
545 Science Drive
Madison, WI 53711
Toll-free: 888.530.0801
Direct: 608.441.2852
Fax: 608.441.2849