Label IT® Nucleic Acid Labeling Kits

Product Name	Quantity*	Product No.
Label IT® CX-Rhodamine Labeling Kit	Full Size	MIR 3100
	Trial Size	MIR 3125
Label IT® Fluorescein Labeling Kit	Full Size	MIR 3200
	Trial Size	MIR 3225
Label IT® Digoxin Labeling Kit	Full Size	MIR 3300
	Trial Size	MIR 3325
Label IT® Biotin Labeling Kit	Full Size	MIR 3400
	Trial Size	MIR 3425
Label IT® Cy™3 Labeling Kit	Full Size	MIR 3600
	Trial Size	MIR 3625
Label IT® Cy™5 Labeling Kit	Full Size	MIR 3700
	Trial Size	MIR 3725
Label IT® DNP Labeling Kit	Full Size	MIR 3800
	Trial Size	MIR 3825
Label IT® TM-Rhodamine Labeling Kit	Full Size	MIR 4100
	Trial Size	MIR 4125

*Each Full Size Kit contains sufficient reagents to Label 100 µg of nucleic acid. Each Trial Size Kit contains sufficient reagents to Label 25 µg of nucleic acid.

1.0 DESCRIPTION

1.1 General Information

Mirus' *Label* IT Nucleic Acid Labeling Technology represents a new class of labeling reagents designed to efficiently and reproducibly attach marker molecules to nucleic acids (DNA or RNA) in a simple one-step reaction within minutes. The *Label* IT Nucleic Acid Labeling Reagents covalently attach CX-rhodamine, fluorescein, digoxin, biotin, Cy™3, Cy™5, dinitrophenol (DNP), and TM-rhodamine labels to nucleic acid bases within DNA or RNA without impacting hybridization performance, and therefore can be used in almost any molecular biology application (excitation and emission wavelengths for each fluorescent *Label* IT Reagent are listed in Table 1). The simplicity of the Labeling reaction, and the fact that only a single reagent is required, suggests that this technology can be widely used in a variety of nucleic acid Labeling applications.

Traditional nonradioactive Labeling methods (random priming, nick translation) are enzyme mediated and thus inherently difficult to control. In addition, these types of reactions generate Labeled products that are not representative of the starting nucleic acid but rather consist of a series of Labeled samples over a variable size range. The Labeling efficiency of these reactions is dictated by the enzyme's ability to incorporate a "Labeled-nucleotide" precursor into a growing nucleic acid chain. This Labeled-nucleotide is not the preferred substrate for the enzyme and may compromise the efficiency of the reaction and introduce a Labeling bias. In contrast, the *Label* IT Labeling reactions are nondestructive, easy to control, and can be scaled up or down by either the size of the reaction or the desired Labeling density.

The supplied standard Labeling protocol will yield Labeling efficiencies of approximately one Label every 20-60 base pairs of double-stranded DNA. Mirus has found that this Labeling density is sufficient to allow sensitive detection for the majority of applications. If there is a need to increase or decrease the density of Labels in the final product, simply modify the ratio of Labeling reagent to nucleic acid during the Labeling reaction or adjust the incubation time of the labeling reaction.

Table 1. Excitation and emission wavelengths For Fluorescent *Label* IT Reagents

Fluorophore	Excitation Wavelength (nm)	Emission Wavelength (nm)
Cy™3	550	570
Cy™5	649	670
CX-Rhodamine	576	597
TM-Rhodamine	546	576
Fluorescein	492	518

1.2 Materials Supplied

Kit Component*	Full Size	Trial Size	Reagent Cap Color
Label IT Reagent	dried pellet	dried pellet	varies with reagent
Reconstitution Solution	100 µl	25 µl	brown
10X Labeling Buffer A	500 µl	125 µl	orange
Denaturation Reagent D1	500 µl	125 µl	blue
Neutralization Buffer N1	500 µl	125 µl	white
G50 Microspin Purification Columns	20	5	N/A

*Extra volume of each kit component is supplied to allow for differences in pipetting devices.

1.3 Materials Required but Not Supplied

If required, detection reagents, including conjugated antibodies, conjugated streptavidin, and chemiluminescent substrates, can be obtained from a variety of commercial sources.

1.4 Storage and Stability

Store the *Label* IT Reagent at –20°C in both its dried pellet and reconstituted form. Cap the Label IT Reagent tightly and avoid exposure to moisture and light. Store all other supplied reagents, including the G50 microspin purification columns, at 4°C. Do not use the G50 microspin columns if they have been stored at –20°C or colder. The *Label* IT Reagent is stable for 6 months after reconstitution. Unreconstituted *Label* IT Reagent and all other reagents are stable for up to 1 year from the date of purchase.

2.0 PROCEDURE

2.1 Labeling Reaction

1. Prior to each use, warm the vial containing the *Label* IT Reagent to room temperature and quick spin to collect the pellet. If using a full size kit, add 100 µl of Reconstitution Solution to the pellet in the vial. If using a trial size kit, add 25 µl of the Reconstitution Solution to the pellet in the vial. To ensure complete reconstitution of the pellet, mix well by gentle pipetting, then perform a quick spin.
2. Prepare the Labeling reaction according to the example shown below. Use water that is both RNase- and DNase-free (molecular biology-grade quality). Add the *Label* IT Reagent last. Use only purified nucleic acid (A_{260}/A_{280} between 1.8 and 2.2) in the Labeling reactions.

Labeling Reaction Example:

Molecular biology-grade H_2O	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**

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 will result in Labeling efficiencies that are appropriate for most applications. If there is a need to increase or decrease the density of Labels in the final product, simply modify the ratio of Labeling reagent to nucleic acid during the Labeling reaction or adjust the incubation time of the Labeling reaction. In addition, the Labeling reaction may be scaled up or down, depending on the amount/volume of nucleic acid to be Labeled. When scaling the Labeling reaction, the amount of *Label* IT Reagent should never constitute more than 20% of the total reaction volume. Ensure that the final concentration of Labeling Buffer A is 1X.

3. Incubate the reaction at 37°C for 1 hour. A quick spin should be performed after 30 minutes of incubation. This will minimize the effect of evaporation and maintain the appropriate concentration of the reaction components.
   NOTE: To denature Labeled DNA for a hybridization application, please see Application Notes (Section 3.0, Part B).
4. Purify the Labeled sample by either ethanol precipitation (see Section 2.2) or using the provided G50 Microspin Purification Columns (see Sections 2.3). Microspin column purification may be particularly beneficial for purification of small amounts (under 1 µg) of nucleic acid. These columns, however, are not recommended if the Labeled sample needs to be quantified by spectrophotometry as gel filtration columns can lead to erroneously high ultraviolet absorbance readings.

2.2 Purification using Microspin Columns

NOTE: The microspin columns are not recommended for nucleic acids under 20 bases in length. Do not use the microspin columns if it is necessary to quantify the Labeled sample after purification. It is generally acceptable to assume 100% recovery of the Labeled nucleic acid following microspin column purification. If the exact quantity of the purified Labeled sample is imperative, use an alternate purification procedure (see Section 2.3, Purification using Ethanol Precipitation).
NOTE: The reaction volume applied to the G50 Microspin Purification Column must be 50 µl. If the reaction volume is less than 50 µl, add 1X Labeling Buffer A to bring the volume to 50 µl. If the reaction volume exceeds 50 µl, split the reaction volume and use 50 µl per column.

A. Centrifuge Conditions

Before using a microspin column, it is important to calculate the speed at which the column should be centrifuged. For a force of 735 x g, the appropriate speed can be calculated from the following formula:

$$rpm = (1000)\,(657/r)^{1/2}$$

where r = radius (in mm) measured from the center of spindle to bottom of the rotor bucket and rpm = revolutions per minute. For example, with a rotor having a radius of 73 mm, the appropriate speed would be 3,000 rpm.

B. Column Preparation

1. Vortex to resuspend the resin in the column.
2. Loosen the cap one-fourth turn then pull out the bottom closure.
3. Place the column in a 1.5 ml screw-cap microcentrifuge tube for support. Alternatively, remove the cap from a flip-top microcentrifuge tube and use this tube as a support.
4. Spin the column for 1 minute at 735 x g (e.g., 3000 rpm in an Eppendorf 5415C variable-speed centrifuge with an 18-position fixed-angle rotor; see Section A above). Start the timer and microcentrifuge simultaneously.
   NOTE: Do not pulse-spin, as this will override the variable speed setting. Use columns immediately after preparation to prevent dehydration of the resin.
5. Discard buffer collected during spin.

C. Sample Application

1. Place the column in a new 1.5 ml microcentrifuge tube.
2. Slowly apply the sample (50 µl) to the top center of the resin. Do not disturb the resin bed.
3. Spin the column at 735 x g for 2 minutes. The purified sample will collect in the bottom of the support tube.
4. Cap the support tube. The Labeled sample is now ready for use. See Section 3.0 for application suggestions.

2.3 Purification using Ethanol Precipitation

NOTE: This purification method is optimal if quantification of the Labeled nucleic acid is necessary. For best recoveries, increase the total volume to 200 µl with 1X Mirus Labeling Buffer A or molecular biology-grade H_2O before adding the sodium chloride and ethanol.

1. If purifying Labeled DNA, add 0.1 volume of 5 M sodium chloride then 2 volumes of ice cold 100% ethanol to the Labeling reaction. Mix and place at –20°C (or colder) for at least 30 minutes. If purifying Labeled RNA, add 0.1 volume of 5 M sodium chloride then 2.5-3 volumes of ice cold 100% ethanol to the Labeling reaction. Mix and place at –20°C (or colder) for at least 30 minutes.
2. Centrifuge at full speed in a microcentrifuge for at least 10 minutes to pellet the Labeled nucleic acid. Aspirate the ethanol, being careful not to disturb the pellet.
   NOTE: Orient the tubes in the microcentrifuge in such a way that it is known where the pellet forms. Small nucleic acid quantities can be invisible to the naked eye.
3. Gently wash the pellet once with 400-500 µl RNase- and DNase-free 70% ethanol (room temperature).
4. After an additional centrifugation (4°C) at full speed for 10 minutes, remove all traces of ethanol with a micropipetter. Do not allow the sample to air dry extensively, as the pellet may become difficult to resuspend.
5. Resuspend the Labeled nucleic acid in 1X Labeling Buffer A or the buffer required for the particular application.

3.0 APPLICATION NOTES

A. Non-Radioactive Hybridization Applications

Conjugated antibodies, conjugated streptavidin, chemiluminescent substrates, and other reagents for biotin, digoxin, DNP and fluorescein detection can be obtained from a variety of sources. For non-radioactive DNA-based membrane hybridization applications, the HybQUEST® Hybridization and Detection Kit (see Related Products, Section 5.0) contains the reagents needed for the preparation, hybridization, and detection of DNP-Labeled samples.

B. Hybridization Reactions using Labeled DNA Samples

For optimal sensitivity and stability of the Labeled DNA sample in hybridization reactions, use the supplied Denaturation Reagent D1 and Neutralization Buffer N1 to denature the Labeled sample prior to any hybridization applications. Do not heat-denature the Labeled DNA probe before this step. Once treated with Denaturation Reagent D1 and Neutralization™ Buffer N1, Labeled DNA samples can be heat denatured as required.

1. Just 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 at least 5 minutes. The Labeled sample is now ready to be used in any hybridization protocol. If the denatured sample is to be used at a later time, store at –20°C and avoid multiple freeze/thaws to maintain the denatured state.

C. Hybridization Reactions using Labeled RNA Samples

For optimal sensitivity and stability of the 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 hydrolize RNA.

D. Fluorescent In Situ Hybridizations

The *Label* IT technology is ideal for use in ISH/FISH applications. Mirus has designed the *Label* IT FISH Kits (see Related Products, Section 5.0) to vividly paint chromosomes or centromeres and reveal the location of specific DNA sequences. The optimized *Label* IT FISH Kits utilize Mirus' *Label* IT technology to prepare superior Labeled probes for hybridization to interphase/metaphase chromosome spreads.

E. Expression Profile Analysis on Microarrays

The *Label* IT µArray^{Biotin} Labeling Kits (see Related Products, Section 5.0) are designed to directly Label mRNA, cDNA, or cRNA for expression profile analysis in microarray applications. These kits provide robust performance demonstrated by high signal to noise ratios, consistent replicates and sensitive detection.

F. *In Vitro* Tracking Experiments

Mirus has developed *Label* IT Tracker™ (DNA tracking) and *Label* IT siRNA Tracker (siRNA tracking) Intracellular Localization Kits (see Related Products, Section 5.0) that provide the necessary reagents to directly Label and transfect either plasmid DNA or siRNA,in an efficient and nondestructive manner. Both subcellular localization and functionality can be monitored in the same experiment following the delivery of the Labeled sample into mammalian cells in culture.

G. *In Vivo* Tracking Experiments

Both subcellular localization and reporter transgene expression can be monitored following the introduction of Labeled plasmid DNA into mammalian cells in vivo. Efficient in vivo delivery of Labeled DNA can be achieved using Mirus' *Trans*IT® In Vivo Gene Delivery System (see Related Products, Section 5.0). This kit is designed for the non-viral delivery of transgenes into laboratory animals via tail vein injection. This kit primarily targets the liver, with lower levels of expression detected in the spleen, lung, heart, and kidneys.

4.0 TROUBLESHOOTING

Suboptimal Nucleic Acid Labeling

- Poor quality of Nucleic Acid
  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 was added to the reaction
  Use 1 µl of *Label* IT Reagent per 1 µg of nucleic acid. See example in Section 2.1 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. Avoid using nucleic acid samples in high salt; NaCl concentrations greater than 50 mM can inhibit the Labeling reaction. Ensure that the final concentration of Buffer A is 1X.
- Improper storage of reagents
  Store both reconstituted and unreconstituted *Label* IT Reagent tightly capped at -20°C. 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 if they have been frozen.
- Nucleic acid pellets were allowed to overdry
  Do not allow the Labeled nucleic acid pellet to dry extensively after ethanol precipitation. Remove traces of the ethanol wash and resuspend immediately in 1X Buffer A or low salt buffer of choice.
- 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 the Application Notes (see Section 3.0). 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.

Determining the Density of Labels on the Nucleic Acid Sample

NOTE: 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 purified Labeled sample may be required to generate significant λ_{max} absorbance readings (for further details, see *Label* IT Reagent Frequently Asked Questions at www.mirusbio.com).

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 demonstrates a distinct reduction in electrophoretic mobility compared to an unlabeled control sample.

For specific questions or concerns, please contact Mirus' Technical Support at 888.530.0801 or techsupport@mirusbio.com.
For a list of citations using Mirus' products, please visit the Technical Resources section of our website (www.mirusbio.com).

5.0 RELATED PRODUCTS

For DNA tracking studies:
Label IT® Tracker™ Intracellular Nucleic Acid Localization Kit (Product # MIR 7010, 7011, 7012, 7013, 7014, 7015)
For RNA tracking studies:
Label IT® siRNA Tracker Intracellular Localization Kit (Product # MIR 7202, 7203, 7204, 7205)
For microarray hybridization studies:
Label IT® µArray^{Biotin} Labeling Kits (Product # MIR 8010 and MIR 8050)
For DNA hybridization studies:
HybQUEST® Complete DNP System (Product # MIR 6000)
HybQUEST® *Label* IT® Kits (Product # MIR 6200, 6300, 6400, 6800)
HybQUEST® Hybridization and Detection Kit (Product # MIR 6010)
In Vivo gene delivery kits*:
TransIT®-*In Vivo* Gene Delivery System (Product # MIR 5100)
Transfection Reagents*:
*Trans*IT®-293 Transfection Reagent (Product # MIR 2700)
*Trans*IT®-3T3 Transfection Kit (Product # MIR 2180)
*Trans*IT®-CHO Transfection Kit (Product # MIR 2170)
*Trans*IT®-COS Transfection Kit (Product # MIR 2190)
*Trans*IT®-Express Transfection Reagent (Product # MIR 2000)
*Trans*IT-HeLaMONSTER® Transfection Kit (Product # MIR 2900)
*Trans*IT®-Insecta Transfection Reagent (Product # MIR 2200)
*Trans*IT®-Jurkat Transfection Reagent (Product # MIR 2120)
*Trans*IT®-Keratinocyte Transfection Reagent (Product # MIR 2800)
*Trans*IT®-LT1 Transfection Reagent (Product # MIR 2300)
*Trans*IT®-LT2 Transfection Reagent (Product # MIR 2400)
*Trans*IT-Neural® Transfection Reagent (Product # MIR 2140)
*Trans*IT®-Oligo Transfection Reagent (Product # MIR 2160)
*Trans*IT®-Prostate Transfection Kit (Product # MIR 2130)
*Trans*IT-TKO® siRNA Transfection Reagent (Product # MIR 2150)
For determination of gene expression efficiency:
Beta-Gal Staining Kit (Product # MIR 2600)
For endotoxin removal from DNA*:
MiraCLEAN® Endotoxin Removal Kit (Product # 5900)

***These products are available in additional sizes.**