Label IT® Nucleic Acid Labeling Kit

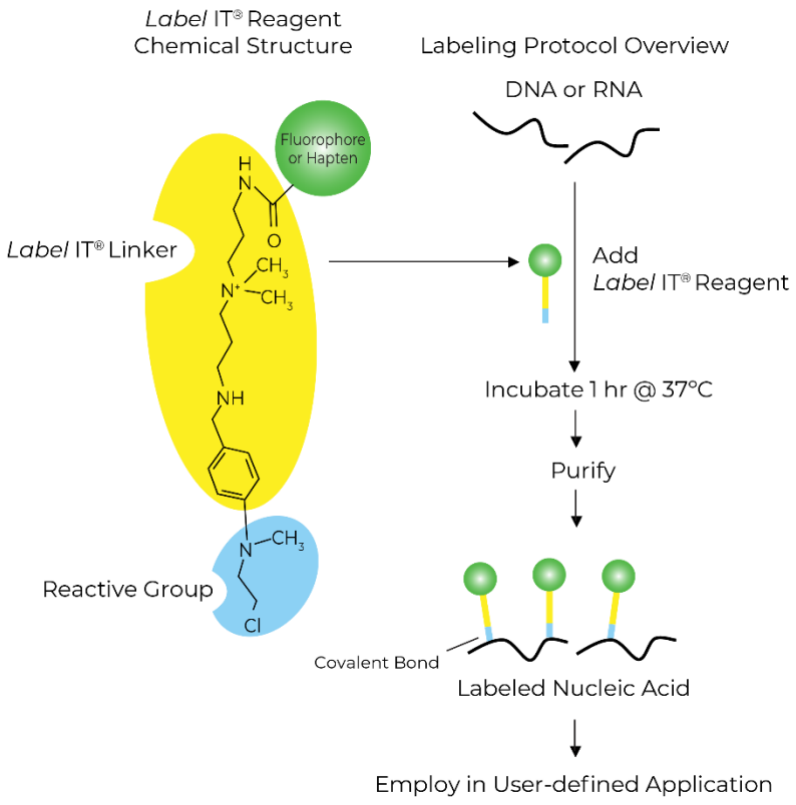
Protocol for MIR 3100, 3125, 3200, 3225, 3400, 3425, 3600, 3625, 3700, 3725, 3900, 3925, 7100, 7125

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

Introduction

The *Label* IT® family of products is designed to easily and efficiently attach detectable molecules to nucleic acids (DNA or RNA) in a simple, scalable reaction. This includes both double and single stranded nucleic acids over 10 nucleotides in length e.g. crRNA, siRNA or ssDNA (see 'Applications' section). The attachment of labels is covalent, non-destructive and enables use in downstream applications such as the tracking of plasmid transfection efficiency.

Following this protocol will achieve a standard labeling density of ~1 label per 20-60 base pairs (i.e. ~1 label per 40-120 bases) when using a 1:1 ratio of *Label* IT® Reagent : nucleic acid (µl:µg). This density is optimal for most applications but can be changed easily if required – see 'Tips for Optimal Labeling' section for additional information.



Specifications

Storage	Store *Label* IT® Reagent (both lyophilized and reconstituted), Reconstitution Solution and 10X Labeling Buffer A at -20°C. Store G50 spin columns at 4°C.
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.
Post-labeling Purification	Post-labeling purification can be carried out either with the G50 spin columns provided or with ethanol precipitation. Using G50 spin columns can interfere with spectrophotometric quantification. It is generally accepted to assume 100% recovery of nucleic acid with spin column purification.

Label IT® Kit Catalog Numbers

Label	Excitation/Emission (nm)	Catalog Number	
		25 µg Size	100 µg Size
Fluorescein	492/518	MIR 3225	MIR 3200
MFP488	501/523	MIR 7125	MIR 7100
Cy™3	550/570	MIR 3625	MIR 3600
CX-Rhodamine	576/597	MIR 3125	MIR 3100
Cy™5	649/670	MIR 3725	MIR 3700
Amine	-	MIR 3925	MIR 3900
Biotin	-	MIR 3425	MIR 3400

Materials Supplied

Kit Component	25 µg Size	100 µg Size
Label IT® Reagent	Lyophilized Pellet	Lyophilized Pellet
Reconstitution Solution	500 µl	500 µl
10X Labeling Buffer A	500 µl	500 µl
G50 Spin Columns	5	20

For Research Use Only

General Labeling Protocol

A. Reconstitute *Label* IT® Reagent

1. Before first use, bring the *Label* IT® Reagent vial to room temperature and centrifuge briefly to collect the dried pellet.
2. Bring the Reconstitution Solution to room temperature and add to the *Label* IT® pellet according to the table below. Mix well and centrifuge to collect the reagent at the bottom.

Kit Size	Reconstitution Solution Volume
25 µg	25 µl
100 µg	100 µl

Allow sufficient time for the Reconstitution Solution to thaw completely.

B. Label Nucleic Acid Sample

1. Prepare the labeling reaction according to the table below in a sterile tube. Add the reagents in the order listed and be sure to add the *Label* IT® Reagent **last**. A 1:1 ratio is suitable for most applications. For more sensitive applications, use 0.5:1 to achieve a lower labeling density, see 'Applications' section for further details.

Component	Ratio of *Label* IT® : Nucleic Acid	
	1:1	0.5:1
DNase & RNase-free water	35 µl	37.5 µl
10X Labeling Buffer A	5 µl	5 µl
1 µg/µl nucleic acid sample	5 µl	5 µl
Label IT® Reagent (add last)	5 µl	2.5 µl
Total Volume	**50 µl**	**50 µl**

A 1:1 ratio will yield a labeling density of ~1 label per 20-60 base pairs (i.e. ~1 label per 40-120 bases). Some applications may require a higher or lower labeling density. For guidance on the adjustment of labeling density, see section 'Tips for Optimal Labeling' or contact: techsupport@mirusbio.com.

2. Incubate the reaction at 37°C for 1 hour. After 30 minutes of incubation, briefly centrifuge the reaction to minimize the effects of evaporation.

C. Purify Samples Using G50 Spin Purification Columns

1. Vortex the column briefly to resuspend the resin. Prepare the column by loosening the cap by one quarter turn and twisting off the bottom closure.
2. Place the column in a sterile 1.5 ml tube and centrifuge for 1 minute at 735xg. Discard the effluent.
3. Repeat step 2.
4. Move the column to a new sterile tube. Slowly apply the 50 µl nucleic acid sample to the column without disturbing the resin bed.
   The volume added in this step must be 50 µl. If lower, make up to volume with 1X Labeling Buffer A. If higher, use 50uL per column i.e. use 3 columns for 150uL.
5. Centrifuge for 2 minutes at 735xg. The purified nucleic acid will collect in the tube.
6. Store the labeled nucleic acid at -20°C and protected from light.

The use of G50 Spin Columns can affect spectrophotometric quantification of nucleic acids. Use with caution or purify by ethanol precipitation to avoid this issue. You can generally assume 100% recovery of nucleic acid from using the G50 spin columns.

Alternative Purification Method: Ethanol Precipitation

If the *Label* IT® reaction volume is less than 100 µl, make up to volume with 1X Labeling Buffer A or nuclease-free water before proceeding.

1. Add 0.1X volume (i.e. 10 µl for 100 µl volume) of 5 M sodium chloride and 2X volume of -20°C pre-chilled 100% ethanol to the labeled nucleic acid. Mix well and incubate for 20 minutes at -20°C.
2. Centrifuge at full speed (>14,000xg) for 30 minutes at 4°C to pellet the nucleic acid.
3. Carefully discard the supernatant without disturbing the pellet.
4. Add 500 µl of room temperature 70% ethanol and centrifuge again at full speed for 15 minutes.
5. Remove all traces of the supernatant without disturbing the pellet. Do not allow the pellet to over-dry (i.e. not longer than 5 minutes unless there are visible ethanol traces. If there are visible ethanol traces, continue to incubate at room temperature until evaporated).
6. Add desired volume of 1X Labeling Buffer A or nuclease-free water and allow to redissolve for 5-30 minutes, followed by gentle pipetting to mix.
7. Store the labeled nucleic acid at -20°C, protected from light.

Tips for Optimal Labeling

Scalability	The *Label* IT® reaction can be scaled up or down as needed. When adjusting volumes, be sure to maintain a 1X final concentration of Labeling Buffer A and the *Label* IT® Reagent should not exceed 20% of total reaction volume.
Labeling Density	A 1:1 ratio of *Label* IT® Reagent (µl): nucleic acid (µg) will yield a standard labeling density of ~1 label per 20-60 base pairs (i.e. ~1 label per 40 to 120 bases). Depending on the application, the labeling density may need adjustment. This can be achieved by: 1) Decreasing/increasing the ratio of *Label* IT® Reagent to nucleic acid, and/or 2) Decreasing/increasing the incubation time at 37°C. Within limits, the relationship between incubation time and labeling density is linear, e.g. to achieve half the labeling density, reduce the incubation time by half.
Determining Labeling Density	A protocol to quantify the labeling density of a labeled sample can be found at mirusbio.com/calculate-nucleic-acid-labeling-density. The relative labeling density can be estimated by dot blot and gel shift analysis.

www.mirusbio.com | techsupport@mirusbio.com | U.S. Toll-Free: 844.647.8724 | Direct: 608.441.2852

Applications

Tracking Transfection Efficiency

Label IT® technology presents an easy and efficient way to track the efficiency of any transfection. Mirus Bio has optimized the labeling density for several common gene delivery applications such that labeling does not interfere with related functional assays:

Approx. Labeling Density (base pairs per label)	*Label* IT® : Nucleic Acid (µl:µg)	Transfection Application or Functional Assay
15-40	1.3:1 to 1.5:1	Gene Knockdown
20-60	1:1	Gene Expression, Gene Knockdown, Probe Hybridization
60-140	0.25:1 to 0.5:1	Gene Expression (Transcription/Translation)
< 60-140	0.1:1 to 0.5:1	*In Vivo* Tracking

In Vivo Tracking

Subcellular localization and reporter transgene expression can be monitored following *in vivo* delivery of labeled nucleic acids. *Label* IT® labeling is compatible with standard fixation procedures.

Please visit the product pages for the *Trans*IT®-EE and *Trans*IT®-QR Hydrodynamic Delivery Solutions. These solutions are designed for hydrodynamic tail vein injection, a method for safe and efficient delivery of nucleic acids into (predominantly) the liver of mice.

Hybridization

To improve sensitivity and stability of labeled DNA probes in hybridization applications, Mirus Bio recommends denaturation of the probe followed by neutralization:

1. Immediately prior to hybridization, add 0.1X volume of Denaturation Buffer (3 M NaOH) (not included) to the DNA probe. Mix well and incubate for 5 minutes at room temperature.
2. Add 0.1X volume of Neutralization Solution (3 M HCl, 1 M Tris) (not included). Mix well and incubate on ice for a minimum of 5 minutes.

Probes can be heat-treated after denaturation. Store denatured probes at -20°C but avoid multiple freeze/thaw cycles to maintain the denatured state. Do **not** use Denaturation and Neutralization Buffers for RNA samples; instead, heat RNA probes at 55-65°C for 10 minutes prior to hybridization.

Conjugation of *Label* IT® Amine with Amine-reactive Dyes

Amine-reactive molecules such as NHS-ester dyes can be further attached to the amine functional group of amine-labeled nucleic acids (after use of MIR 3900 and MIR 3925):

1. Combine at least 5 µg of purified amine-labeled nucleic acid with 10 mM of the NHS-ester compound (in anhydrous DMSO) and freshly prepared 100 mM $NaHCO_3$ pH 8.5. Incubate at room temperature for one hour. Protect fluorescent compounds from light.
2. Purify labeled nucleic acids using G50 spin columns or ethanol precipitation as detailed in the 'General Labeling Protocol' section above.

www.mirusbio.com | techsupport@mirusbio.com | U.S. Toll-Free: 844.647.8724 | Direct: 608.441.2852

Troubleshooting

Poor/Suboptimal Nucleic Acid Labeling	
Poor Nucleic Acid Quality	- Ensure that starting material is free from contaminants and has an $A_{260/280}$ between 1.8 to 2.2. - Use DNase- and RNase-free consumables and handling procedures.
Improper Scaling	- Maintain the appropriate ratio of *Label* IT® Reagent to nucleic acid when scaling, e.g. 1:1. - Keep the volume of *Label* IT® Reagent to < 20% of total reaction volume. - Ensure that the final concentration of Labeling Buffer A is 1X. - Avoid using high-salt nucleic acid buffers. NaCl concentrations exceeding 50 mM can be inhibitory.
Improper Storage	- Keep G50 spin columns at 4°C. Do **not** use if frozen. - Keep all other *Label* IT® Reagents at -20°C, protected from light.
Labeling Density	- For some applications, using the standard labeling density of 1 label per 20-60 base pairs may be inadequate. Adjust accordingly; see 'Tips for Optimal Labeling' and 'Applications' sections.

Reagent Agent®

Unsure about which transfection reagent you should use? Consult Reagent Agent®.

mirusbio.com/ra