Label IT® Nucleic Acid Modifying Kit

Protocol for MIR 3900, 3925

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

INTRODUCTION

The *Label* IT® Nucleic Acid Modifying Kit is designed to efficiently and reproducibly attach amine functional groups to any nucleic acid in a one-step, scalable reaction. Unlike enzyme mediated labeling methods, the *Label* IT® Technology covalently attaches an amine to bases within any DNA or RNA molecule without dramatically altering the starting material or hindering downstream hybridization performance. Potential downstream applications for *Label* IT® Nucleic Acid Modifying Kit include conjugation of amine modified nucleic acids to activated carboxylic acid groups on proteins, labeling amine modified nucleic acids with NH_2-reactive dyes and attachment of amine modified nucleic acids to NH_2- reactive glass surfaces in microarray applications.

SPECIFICATIONS

Storage	Store *Label* IT® Reagent at -20°C in both dried and reconstituted forms. Store Reconstitution Solution, 10X Buffer A, Denaturation Reagent D1 and Neutralization Buffer N1 at -20°C. Store G50 microspin purification 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	MIR 3900 contains sufficient reagents to label 100 µg of nucleic acid. MIR 3925 contains sufficient reagents to label 25 µg of nucleic acid.

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

MATERIALS

Materials Supplied

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

Kit Component	MIR 3900	MIR 3925	Reagent Cap Color
Label IT® Modifying Reagent	Dried pellet	Dried pellet	Red
Reconstitution Solution	100 µl	100 µl	Brown
10X 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 columns	5 columns	N/A

Materials Required, but Not Supplied

- Molecular biology-grade water
- Nucleic acid sample (starting material)
- Optional: Materials for ethanol purification
- Optional: NH_2 secondary conjugates or detection reagents

For Research Use Only

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 Buffer A and ensure that the *Label* IT® Reagent does NOT constitute greater than 20% of total reaction volume.
- **Labeling ratio.** The 0.5:1 (v:w) ratio of *Label* IT® Modifying Reagent to nucleic acid outlined in this protocol typically results in a labeling density suitable for most applications. Ratios between 0.2:1 and 0.8:1 result in labeling efficiencies that are appropriate 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*. It may be necessary to titrate the level of modification for your particular application. To modify the labeling density of the sample, increase or decrease the amount of *Label* IT® Reagent used in the reaction or adjust the reaction incubation time.
- **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 microspin column purification. However, if the labeled sample will be quantified by spectrophotometry, purification by ethanol precipitation is recommended as gel purification leads to erroneously high ultraviolet A_{260} readings.
- **Determining the labeling density of the nucleic acid sample.** A labeling density of 1 label per every 35-95 bases of nucleic acid can be expected if using a 0.5:1 (v:w) ratio of *Label* IT® Reagent to nucleic acid. Please visit the Mirus Bio *Label* IT® FAQ and Tips from the Bench pages for information on determing the exact labeling density of your sample. The relative density of labels on purified, labeled nucleic acid can be estimated by one of the following methods:
    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 0.5:1 (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. Store the reconstituted *Label* IT® Reagent at -20°C and protect from moisture.

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

1. Before the first use, warm the *Label* IT® Reagent 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 No.	Reconstitution Solution Volume
MIR 3925	25 µl to each *Label* IT® pellet
MIR 3900	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.

Increase or decrease the amount of *Label* IT® Reagent in the reaction or adjust the reaction incubation time to modify the labeling density. Ratios between 0.2:1 and 0.8:1 (v:w) result in labeling efficiencies appropriate for most applications.

Standard Nucleic Acid Modifying Reaction	
DNase-, RNase-free (molecular biology-grade) water	37.5 µl
10X Buffer A	5 µl
1 mg/ml nucleic acid sample	5 µl
Label IT® Modifying Reagent, Amine	2.5 µl
Total Volume	**50 µl**

NOTE: This example labels 5 µg of nucleic acid at a 0.5:1 (v:w) ratio of *Label* IT® Reagent to nucleic acid, resulting in a labeling density 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. Ensure that the final concentration of Buffer A is 1X and the *Label* IT® Reagent does not exceed 20% of the total reaction volume.

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 to maintain the appropriate concentration of the reaction components.

D. Purification using the 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 × 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 Buffer A. If the volume exceeds 50 µl, split the reaction and use 50 µl per column.
7. Centrifuge the column at 735 × 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 and moisture.

E. Purification using ethanol precipitation (Alternative Method)

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

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 Buffer A or molecular biology-grade water before adding sodium chloride and ethanol.

1. Add 0.1 volume of 5 M 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 × g) in a refrigerated microcentrifuge for 15-30 minutes to pellet the labeled nucleic acid. Once pelleted, gently remove the ethanol with a micropipette; 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 micropipette. 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 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. Conjugation of Modified Nucleic Acids with Amine-Reactive Dyes

Amine-modified nucleic acids can be conjugated to amine-reactive dyes such as NHS-ester dyes for additional applications (e.g. fluorescent tracking of nucleic acids *in vitro* or *in vivo*). The following is an example reaction:

1. Combine at least 5 µg of purified *Label* IT® Amine-modified DNA with 10 mM of the succinimidyl ester fluorophore of choice (prepared in anhydrous DMSO) and 100 mM $NaHCO_3$ (pH ~8.5, freshly prepared). Incubate for one hour at room temperature in the dark.
2. Following subsequent purification, it is possible to determine the exact labeling density of your sample by following instruction on Mirus Bio *Label* IT® FAQ or Tips from the Bench pages.

NOTE: Further applications include conjugation of amine-modified nucleic acids to proteins/peptides using activated carboxylic acid groups on the protein and attachment of amine-modified nucleic acids to amine reactive glass surfaces in microarrays.

B. *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. To identify the ideal transfection reagent for delivering labeled DNA/siRNA to your cell type, see the Related Products Section (Page 7) or visit the Reagent Agent Transfection Database at mirusbio.com/reagent-agent.

C. *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.

D. Hybridization Reaction Using Modified DNA Samples

Amine-modified nucleic acid may be custom-labeled with dyes for use in hybridization reactions (see Section A above). For optimal sensitivity and stability of the DNA probe 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:

Use RNase and DNase-free components.

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.

E. 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.

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 3) 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 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 over-dried (after ethanol precipitation)	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 5). 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® siRNA Tracker™ 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
- *Trans*IT®-QR Hydrodynamic Delivery Solution

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

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