

Label IT® Nucleic Acid Modifying Kit

Quick Reference Protocol

Instructions for MIR 3900, 3925

Full protocol, SDS and Certificate of Analysis available at mirusbio.com/3900



SPECIFICATIONS

Storage	Store Label IT® Modifying Reagent at -20°C in both dried and reconstituted form. Store Reconstitution Solution, 10X Labeling Buffer, Reagent D1 and 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 Sizes and Usage	The Full Size Kit contains sufficient reagent to label 100-200 µg nucleic acid. The Trial Size Kit contains sufficient reagent to label 25-50 µg nucleic acid.

▶ Label IT® NUCLEIC ACID MODIFYING REACTION



Full protocol and additional documentation available at mirusbio.com/3900

The Label IT® technology is designed to efficiently and reproducibly attach marker molecules or functional groups to nucleic acids in a simple and tunable reaction. With Label IT® Nucleic Acid Modifying Kits, NH₂ functional groups are rapidly and covalently attached to nucleic acids for use in a variety of applications.

A. Prepare and reconstitute the Label IT® Nucleic Acid Modifying Reagent.

1. Warm the Label IT® Nucleic Acid Modifying Kit to room temperature.
2. Briefly centrifuge the Label IT® Reagent to collect the lyophilized pellet.
3. Add the appropriate volume of Reconstitution Solution to the reagent pellet and mix well:
 - If using the Full Size Kit (MIR 3900), reconstitute with 100 µl of Reconstitution Solution.
 - If using the Trial Size Kit (MIR 3925), reconstitute with 25 µl of Reconstitution Solution.

NOTE: Once reconstituted, the Modifying Reagent (MW 486.8 g/mol) is at a concentration of 1 mg/ml.

B. Prepare the labeling reaction according to the example below. Add the Label IT® Reagent last.

Labeling Reaction Example:

Molecular Biology-grade H ₂ O	37.5 µl
10X Labeling Buffer A	5 µl
1 mg/ml nucleic acid sample	5 µl
Label IT® Modifying Reagent	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 Labeling 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 maintain the appropriate concentration of the reaction components.

D. Purify the labeled nucleic acid sample using G50 Microspin Purification Columns.

NOTE: If the labeled sample will be quantified by spectrophotometry, purification by EtOH precipitation is recommended as G50 Microspin Column purification can lead to erroneously high ultraviolet A260 readings. It is generally acceptable to assume 100% recovery of the labeled nucleic acid following column purification.

1. Vortex to resuspend the resin in the column.
2. Loosen the cap 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 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. Purified sample will collect in the microfuge tube.
8. Discard the column and cap the microcentrifuge tube. The labeled sample is now ready for use.
9. Store the labeled nucleic acid on ice for immediate use or at -20°C for long-term storage.

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▶ Label IT® NUCLEIC ACID LABELING APPLICATIONS



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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 NaHCO₃ (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 instructions in Label IT® [Frequently Asked Questions](#) or [Tips from the Bench](#).

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. 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 transfection reagent for Labeled DNA/siRNA delivery to your cell type, visit the [Reagent Agent Transfection Database](#) at www.mirusbio.com.

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 TransIT®-EE and TransIT®-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 Reactions using Modified DNA Samples

The NH₂-modified nucleic acid may be custom-labeled with dyes for use in hybridization reactions. For optimal sensitivity and stability of 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:

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 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.

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