

Label IT[®] Nucleic Acid Modifying Kits
 Product # MIR 3900, MIR 3925, MIR 4000, MIR 4025

<i>Label IT[®] Amine Modifying Kit</i>	MIR 3900	for modifying at least 100 µg nucleic acid
	MIR 3925	for modifying at least 25 µg nucleic acid
<i>Label IT[®] COOH Modifying Kit</i>	MIR 4000	for modifying at least 100 µg nucleic acid
	MIR 4025	for modifying at least 25 µg nucleic acid

1.0 INTENDED USE

Label IT[®] Nucleic Acid Modifying Kits are supplied with sufficient reagents to modify at least 25 µg or 100 µg of DNA or RNA. Each of the kits facilitate nondestructive covalent attachment of NH₂ or COOH functional groups to DNA or RNA within minutes.

2.0 DESCRIPTION

2.1 General Information

Mirus Bio's *Label IT[®] Nucleic Acid Modifying* reagents comprise a new line in our selection of labeling reagents. All of the *Label IT[®] Reagents* are designed to efficiently and reproducibly attach marker molecules or functional groups to nucleic acids in a simple one-step reaction. The ability to modify DNA and RNA simply and reproducibly with NH₂ or COOH functional groups represents a large technological step forward in the nucleic acid field. With *Label IT[®] Nucleic Acid Modifying Reagents*, the functional groups can be covalently attached to any nucleic acid species within minutes. Furthermore, unlike enzyme mediated labeling methods, the simplicity of this chemical labeling reaction allows one to directly control the level of nucleic acid modification.

2.2 Potential Applications

Label IT[®] Amine Modifying Kit

- Conjugation of modified nucleic acids to proteins/peptides using activated carboxylic acid groups on the protein
- Labeling modified nucleic acid with amine-reactive dyes (e.g. NHS ester, sulfonyl chloride and isothiocyanate dyes; see below)
- Attachment of modified nucleic acids to amine reactive glass surfaces in microarray applications.

Label IT[®] COOH Modifying Kit

- Conjugation of modified nucleic acid to proteins/peptides using water-soluble carbodiimide chemistry
- Labeling modified nucleic acids with hydrazine or amine dyes (see below)
- Attachment of modified nucleic acid to surfaces. For example:
 1. Hydrazide coated plates
 - a. Carbo-BIND[MC1][™] covalent assay plates and strips (Corning-Costar; Acton, MA)
 - b. Avidplate-HZ microplates (UniSyn Technologies; Tustin, CA)
 2. Hydrazide coated microspheres
 - a. Magnetic porous glass (MPG) beads/hydrazide (CPG, Inc.; Lincoln Park, NJ)
 - b. Hydrazide latex particles (Magsphere, Inc.; Pasadena, CA)

2.3 Advantages of *Label IT*[®] Modifying Kits Over Existing Methods

- Simple, one-step reaction: Simply mix modifying reagent and nucleic acid together and incubate at 37°C for 30-60 minutes.
- Modify any nucleic acid: Reagents work equally well with DNA (double stranded, single stranded, supercoiled or linear) or RNA.
- Nondestructive: DNA or RNA substrate remains intact after the reaction whether you are starting with supercoiled plasmid DNA, large linear fragments or oligonucleotides, thus allowing quantitative recovery of modified product.
- Highly efficient: *Label IT*[®] Reagents can exceed one label per every ten nucleotides of nucleic acid if necessary. The protocol allows control over the level of nucleic acid modification.
- Reproducible: In contrast to enzyme-mediated methods, the chemical reactions with *Label IT*[®] Reagents are very reproducible.

Materials Supplied

	Standard Size (for modifying at least 100 µg)	Trial size (for modifying at least 25 µg)	Reagent Cap Color
<i>Label IT</i> [®] Modifying Reagent	100 µg dried pellet	25 µg dried pellet	varies with reagent
Reconstitution Solution*	110 µl	35 µl	clear
10X Labeling Buffer A	500 µl	125 µl	lilac
Denaturation Buffer D1	500 µl	125 µl	blue
Neutralization Buffer N1	500 µl	125 µl	clear
G50 Microspin Purification Columns**	20	5	N/A

* An extra 10 µl of Mirus Reconstitution Solution is supplied in each size kit to allow for slight variations in pipetting devices.

** Enough columns are provided for 20 or 5 standard reactions at 5 µg each.

Storage and Stability

The *Label IT*[®] Reagent should be stored at -20°C in both its dried pellet and reconstituted form. Store all other supplied reagents, including the columns, at 4°C. 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.

3.0 PROCEDURE

3.1 Modifying Reaction

1. For a standard size kit, add 100 µl of Mirus Reconstitution Solution to the *Label IT*[®] Reagent pellet (for a trial size kit, add 25 µl of the Reconstitution Solution to the dried pellet). To ensure reconstitution of the pellet, mix well by pipetting up and down repeatedly. Once reconstituted, the modifying reagent is at a concentration of 1 mg/ml (the molecular weight of the Amine reagent is 270, while the COOH reagent has a molecular weight of 797). Store at -20°C and protect from moisture.
2. Prepare your reaction according to the example shown below. Ensure that the distilled H₂O being used is of molecular biology-grade quality. Be sure to add the *Label IT*[®] Reagent last. We recommend a mass ratio between 0.2:1 and 0.8:1 for the reactions. Please note that the level of modification is generally linear over this range. It may be necessary to titrate the level of modification ("mass ratio") for your particular application.

For example, a reaction using a 0.5:1 ratio (mass *Label IT*[®] Reagent:mass nucleic acid) with 5 µg nucleic acid would require 2.5 µl (2.5 µg) *Label IT*[®] Reagent (in a final volume of 50 µl).

dH ₂ O	37.5 µl
10X Mirus Labeling Buffer A	5 µl
1 mg/ml nucleic acid solution	5 µl
<i>Label IT</i> [®] Reagent	2.5 µl (add <i>Label IT</i> [®] Reagent last)

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 reaction at 37°C for 1 hour.
4. Remove unreacted reagent from the modified nucleic acid by either ethanol precipitation or using one of the provided G50 Microspin Purification Columns (see Sections 3.2 and 3.3). Microspin Column purification may be particularly beneficial for purification of small amounts (under 1 µg) of nucleic acid.
NOTE: The reaction volume applied to the Microspin Purification Column must be 50 µl. If the reaction volume is less than 50 µl, add 1X Mirus Labeling Buffer A to bring the volume to 50 µl. If the reaction volume exceeds 50 µl, split the reaction volume and use more than one column, keeping in mind that the volume added to each column must equal 50 µl.
5. Store the purified, modified nucleic acid at -20°C.
NOTE: An aliquot of the modified nucleic acid can be run alongside unmodified nucleic acid on an agarose gel to assess the modification efficiency. Modified nucleic acid displays a marked reduction in electrophoretic migration, predominantly due to the covalent attachment of the functional groups. Also, please see the assays for the detection of the amine/COOH functional groups in the Applications section of this guide (Section 4.0.C).

3.2 Microspin Column Procedure

NOTE: This protocol is not recommended for nucleic acids under 20 bases in length. Assume 100% recovery of modified DNA – spectrophotometric quantification is generally not accurate after gel filtration due to erroneous readings.

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:

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

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 and snap off 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. Pre-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.

C. Sample Application

1. Place the column in a new 1.5 ml microcentrifuge tube and slowly apply the sample (50 μ l) to the top center of the resin, being careful not disturb the resin bed.
2. Spin the column at 735 x g for 2 minutes. The purified sample will collect in the bottom of the support tube.
3. Cap the support tube. The modified nucleic acid is now ready for use. See Section 4.0 for application notes.

3.3 Purification of Modified Nucleic Acid by Ethanol Precipitation

1. For DNA, add 0.1 volume of 5 M sodium chloride and 2 volumes of ice cold 100% ethanol to your reaction. Mix and place in a -20°C (or colder) freezer for at least 10 minutes. For RNA, add 0.1 volume of 5 M sodium chloride and 2.5 volumes of ice cold 100% ethanol to your reaction. Mix and place in a -20°C (or colder) freezer for at least one hour.

NOTE: For smaller reaction volumes (<20 μ l), bring the volume up to 200 μ l with 1X Labeling Buffer A or H_2O before adding the sodium chloride and ethanol.

2. Centrifuge at full speed in a microcentrifuge for 10 minutes to pellet the modified nucleic acid. Aspirate the ethanol, being careful not to disturb the pellet.
3. Gently wash the pellet once with 70% ethanol, being careful not to disturb the pellet. After an additional centrifugation at full speed, remove all traces of ethanol with a micropipetter. Do not allow the sample to air dry extensively, as the pellet will become extremely difficult to resuspend. Resuspend the modified nucleic acid in 1X Labeling Buffer A or your preferred buffer.

NOTE: If the amount of nucleic acid is less than 1 to 2 μ g, be sure to orient the precipitate-containing tubes in the microcentrifuge in such a way that you will know where the pellet forms. Nucleic acid quantities this small can be invisible to the naked eye.

4.0 APPLICATION NOTES

A. For All Hybridization Reactions Using Modified DNA as a Probe

The NH_2 - or COOH -modified nucleic acid may be custom-labeled with dyes for use in hybridization reactions. For optimal sensitivity and stability of the DNA probe in hybridization reactions, Mirus Bio recommends using the supplied Denaturation Reagent D1 prior to any hybridization applications. Do not heat-denature the modified DNA probe.

1. Just prior to the hybridization, add 0.1 volume of Denaturation Reagent D1 to the probe DNA.
2. Mix well and incubate for 5 minutes at room temperature. Quickly chill on ice.
3. Add 0.1 volume of Neutralization Buffer N1. Mix well and incubate on ice for at least for 5 minutes. The probe is now ready to be used in any hybridization protocol. If the denatured probe is to be used at a later time, it should be stored at -20°C to maintain the denatured state.

B. For All Hybridization Reactions Using Modified RNA as a Probe

The NH_2 - or COOH -modified nucleic acid may be custom-labeled with dyes for use in hybridization reactions. For optimal sensitivity and stability of the DNA probe in hybridization reactions, Mirus recommends using the supplied Denaturation Reagent D1 prior to any hybridization applications. Do not heat-denature the modified DNA probe.

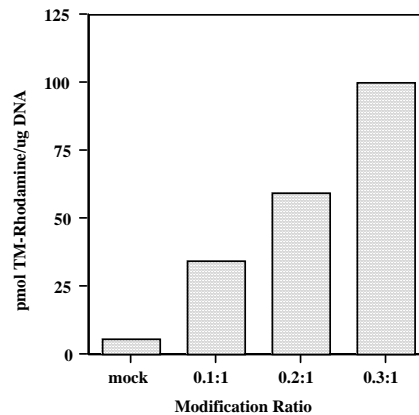
For optimal sensitivity and stability of the RNA probe, Mirus Bio recommends denaturing the RNA by heating at 55- 65°C for 10 minutes prior to any hybridization applications. Do not denature the modified RNA probe with Denaturation Reagent D1, as alkaline conditions can destroy RNA.

C. Assay for Functional Amine Groups on Modified DNA - Reaction with Succinimidyl Esters

React at least 5 μ g of purified *Label IT*[®] Amine-modified DNA, with replicates, with 10 mM of the succinimidyl ester fluorophore of choice (prepared in anhydrous DMSO) and 100 mM NaHCO_3 (pH ~ 8.5, freshly prepared) for one hour at room temperature in the dark. Following subsequent purification, determine the labeling efficiency using either spectrophotometric or fluorimetric analysis. To validate the data, it is important to include appropriate controls:

- buffer + fluorophore reagent
- unmodified DNA + fluorophore reagent

Figure 1

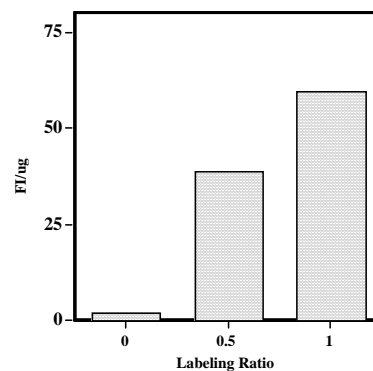


Plasmid DNA was reacted with the *Label IT*[®] Amine reagent at the ratios (w:w) of Modifying Reagent to DNA indicated and purified by ethanol precipitation. Five μg of the modified DNA, in triplicates, was reacted with 10 mM NHS ester TM-rhodamine (in DMSO, Invitrogen), and 100 mM NaHCO_3 (pH ~ 8.5, freshly prepared) for one hour at room temperature in the dark. The DNA was again ethanol precipitated, washed extensively and assayed for labeling efficiency using absorbance at λ_{MAX} (546 nm).

D. Assay for Functional COOH Groups on Modified DNA - Reaction with Lissamine[™] Rhodamine B Ethylenediamine

Ethanol precipitate at least 5 μg of COOH-modified DNA and resuspend the pellet in 40 to 50 μl 25 mM MES buffer, pH 6-6.5. Add 5 μl of 10 $\mu\text{g}/\mu\text{l}$ EDC (1-ethyl-3-(3-dimethylaminopropyl carbodiimide hydrochloride; Aldrich, prepared immediately before use in 25 mM MES buffer, pH 6-6.5) and 5 μl Lissamine[™] rhodamine B ethylenediamine reagent (Invitrogen, prepare 10 mg/ml in methanol, store aliquoted at -20°C). Mix well and incubate at room temperature for several hours, protected from light. Ethanol precipitate the DNA again and resuspend each pellet in 25 mM MES buffer, pH 6 - 6.5. Including the appropriate controls, estimate labeling efficiency using either spectrophotometric or fluorimetric analysis.

Figure 2



Plasmid DNA (5.6 kb) was reacted with *Label IT*[®] COOH reagent at the ratios (w:w) of Modifying Reagent to DNA indicated and purified by ethanol precipitation. Ten μg of the modified DNA, in triplicate, was reacted with 1 mg/ml EDC (1-ethyl-3-(3-dimethylaminopropyl carbodiimide HCl, freshly prepared in 25 mM MES, pH 6-6.5) and 1 mg/ml Lissamine[™] Rhodamine B ethylenediamine (Molecular Probes) for 3 h at room temperature, protected from light. The DNA was again ethanol precipitated, washed extensively and assayed for labeling efficiency.

5.0 Related Products**For endotoxin removal from DNA:**

MiraCLEAN[®] Endotoxin Removal Kit (Product #5910, 5900)

For DNA tracking studies:

Label IT[®] Tracker[™] Intracellular Nucleic Acid Localization Kit (Product # MIR7010,7011,7012,7013,7014,7015)

For RNA tracking studies:

Label IT[®] siRNA Tracker Intracellular Localization Kit with *TransIT*-TKO[®] Transfection Reagent
(Product # MIR 7200,7201,7202,7203,7204,7205)

Label IT[®] siRNA Tracker Intracellular Localization Kit with *TransIT*[®]-siQUEST[™] Transfection Reagent
(Product # MIR 7206,7207,7208,7209,7210,7211)

Label IT[®] siRNA Tracker Intracellular Localization Kit (Product # MIR 7212,7213,7214,7215,7216,7217)

For microarray hybridization studies:

Label IT[®] μ Array Biotin Labeling Kits (Product # MIR 8010 and MIR 8050)

Label IT[®] μ Array Dual Labeling Kits (Product # MIR 8105 and MIR 8125)

Label IT[®] μ Array Cy[™]3/Cy[™]5 Labeling Kits (Product # MIR 8205 and MIR 8225)

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)

Labeled Delivery Controls:

Label IT[®] Plasmid Delivery Control (MIR 7904, 7905, 7906, 7907)

Label IT[®] RNAi Delivery Control (MIR 7900, 7901, 7902, 7903)

In Vivo Gene Delivery Kits:*

TransIT[®]-*In Vivo* Gene Delivery System (Product # MIR 5100)

TransIT[®]-EE Hydrodynamic Delivery Solution (Product # MIR 5340)

TransIT[®]-EE Hydrodynamic Delivery Starter Kit (Product # MIR 5310)

TransIT[®]-QR Hydrodynamic Delivery Solution (Product # MIR 5240)

TransIT[®]-QR Hydrodynamic Delivery Starter Kit (Product # MIR 5210)

Mirus Bio Reagents are covered by United States Patent No. 5,744,335; 5,965,434; 6,180,784; 6,383,811; 6,593,465 and patents pending.

The performance of this product is guaranteed for one year from the date of purchase if stored and handled properly.

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