Label IT® Nucleic Acid Labeling Kits

Quick Reference Protocol

Instructions for MIR 3100, 3125, 3200, 3225, 3300, 3325, 3400, 3425, 3600, 3625, 3700, 3725, 3800, 3825, 4100, 4125, 7100, 7125

Full protocol, SDS and Certificate of Analysis available at mirusbio.com/3100

SPECIFICATIONS

Storage	Store *Label* IT® 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	Each Full Size Kit contains sufficient reagents to label 100 µg nucleic acid. Each Trial Size Kit contains sufficient reagents to label 25 µg nucleic acid.

▸ *Label* IT® NUCLEIC ACID LABELING REACTION

Full protocol and additional documentation available at ***mirusbio.com/3100***

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

1. Warm the *Label* IT® Nucleic Acid Labeling Kit to room temperature before use.
2. Briefly centrifuge the *Label* IT® Reagent to collect the lyophilized pellet.
3. Reconstitute the reagent pellet with the appropriate volume of Reconstitution Solution:
   - If using a Full Size Kit (e.g. MIR 3100), reconstitute with 100 µl of Reconstitution Solution.
   - If using a Trial Size Kit (e.g. MIR 3125), reconstitute with 25 µl of Reconstitution Solution.

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

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 Reaction 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 and results in a labeling density of 1 label per every 20-60 base pairs. Increase or decrease the amount of Label IT® Reagent in the reaction or adjust the reaction incubation time to modify the labeling density (see Label IT® Nucleic Acid Labeling Applications section). The Label IT® Reagent should never 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 evaporation.

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.

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. Discard the buffer collected during the spin. Repeat this process one additional time to remove residual liquid.
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 sample on ice for immediate use or at -20°C long-term, protected from light.

▸ Determine the Nucleic Acid Sample Labeling Density

A labeling density of 1 label per every 20-60 base pairs of nucleic acid can be expected if using a 1:1 (w:v) ratio of *Label* IT® Reagent to nucleic acid in the protocol detailed above. If it is necessary to determine the exact labeling density of your sample, see instructions in the *Label* IT® Frequently Asked Questions or Tips from the Bench.

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▸ *Label* IT® NUCLEIC ACID LABELING APPLICATIONS

Full protocol and additional documentation available at ***mirusbio.com/3100***

Table 1: *Label* IT® Nucleic Acid Labeling Kits and excitation/emission wavelengths:

Label IT Product Name	Excitation Wavelength (nm)	Emission Wavelength (nm)	Product No.
Label IT® Nucleic Acid Labeling Kit, CX-Rhodamine	576	597	MIR 3100, MIR 3125
Label IT® Nucleic Acid Labeling Kit, Fluorescein	492	518	MIR 3200, MIR 3225
Label IT® Nucleic Acid Labeling Kit, Digoxin	n/a	n/a	MIR 3300, MIR 3325
Label IT® Nucleic Acid Labeling Kit, Biotin	n/a	n/a	MIR 3400, MIR 3425
Label IT® Nucleic Acid Labeling Kit, Cy®3	550	570	MIR 3600, MIR 3625
Label IT® Nucleic Acid Labeling Kit, Cy®5	649	670	MIR 3700, MIR 3725
Label IT® Nucleic Acid Labeling Kit, DNP	n/a	n/a	MIR 3800, MIR 3825
Label IT® Nucleic Acid Labeling Kit, TM-Rhodamine	546	576	MIR 4100, MIR 4125
Label IT® Nucleic Acid Labeling Kit, MFP488	501	523	MIR 7100, MIR 7125

A. *In Vitro* Tracking Experiments

Subcellular localization and target gene functionality can be monitored in the same experiment following the delivery of the *Label* IT® labeled sample into mammalian cells in culture. Lower labeling densities (e.g. 0.25:1 - 0.5:1) are recommended for applications for which the labeled DNA will be used for *in vitro* gene expression studies. To modify labeling density of the sample, simply increase or decrease the amount of *Label* IT® Reagent used in the reaction or adjust the reaction incubation time, as the labeling reaction is linear over the first three hours of incubation at 37°C.

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 (respectively) for *in vitro* and *in vivo* 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.

B. *In Vivo* Tracking Experiments

Subcellular localization and reporter transgene expression can be monitored following the introduction of labeled nucleic acids into mammalian cells *in vivo*. Lower labeling densities (e.g. 0.1:1 – 0.5:1) are recommended for applications for which labeled DNA will be used for *in vivo* gene expression studies. To modify the labeling density of the sample, simply increase or decrease the amount of *Label* IT® Reagent used in the reaction or adjust the reaction incubation time, as the labeling reaction is linear over the first three hours of incubation at 37°C.

The *Trans*IT®-EE and *Trans*IT®-QR Hydrodynamic Delivery Solutions are designed specifically for 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.

C. Hybridization Reactions using Labeled DNA Samples

For optimal sensitivity and stability of labeled DNA samples 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. See the Full Protocol for further details.

D. 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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