Label IT® Tracker™ Intracellular Nucleic Acid Localization Kits

Quick Reference Protocol

Instructions for MIR 7020, 7021, 7022, 7023, 7024, 7025

Full protocol, SDS and Certificate of Analysis available at mirusbio.com/7020

SPECIFICATIONS

Storage	Store *Label* IT® Tracker™ Reagent at –20°C in both dried and reconstituted form. Store Tracker Reconstitution Solution and 10X Labeling Buffer A at –20°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.

Label IT® TRACKER™ PLASMID LABELING REACTION

Full protocol and additional documentation available at ***mirusbio.com/7020***

A. Prepare and reconstitute the *Label* IT® Tracker™ Reagent.

1. Warm *Label* IT® Tracker ™ Reagent to room temperature and briefly centrifuge to collect the pellet.
2. For first use only, add 50 µl of Tracker Reconstitution Solution to the pellet and mix well.

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

Labeling Reaction Example:	
Molecular Biology-grade H_20	37.5 µl
10X Labeling Buffer A	5 µl
1 mg/ml plasmid DNA	5 µl
Label IT® Tracker™ Reagent	2.5 µl
Total Volume:	**50 µl**

NOTE: *This example labels 5 µg plasmid DNA at a 0.5:1 (v:w) ratio of Label IT® Tracker™ Reagent to DNA and results in a labeling density of 1 label per 60-140 base pairs. For DNA tracking applications, ratios ranging from 0.25:1 to 1:1 (v:w) are recommended. To modify the labeling density, increase or decrease the amount of Label IT® Reagent in the reaction or adjust the reaction incubation time. The Label IT® Reagent should never exceed 20% of the total reaction volume.*

C. Incubate the reaction at 37°C for I 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. Remove unreacted *Label* IT® Tracker™ Reagent by ethanol precipitating the Labeled plasmid.

NOTE: For labeling reaction volumes <100 µl, bring the volume to 100 µl with 1X Labeling Buffer A or sterile 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 x g) in a refrigerated microcentrifuge for 15-30 minutes to pellet the labeled DNA. Gently remove the ethanol with a micropipetter; do not disturb the pellet.
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 micropipetter. DO NOT allow the sample to dry longer than 5 minutes as the pellet may become difficult to resuspend.
5. Resuspend labeled DNA in an appropriate volume of 1X Labeling Buffer A or sterile water.
6. If an exact concentration is required, quantify the purified, labeled DNA on a spectrophotometer and dilute to the desired working concentration.
7. Store the purified, labeled DNA on ice for immediate use or at –20°C for long-term storage. Protect the labeled sample from light.

Determine the Nucleic Acid Sample Labeling Density

A labeling density of 1 label per every 60-140 base pairs can be expected if using a 0.5:1 (v:w) ratio of *Label* IT® Tracker™ Reagent to plasmid DNA in the protocol detailed above. If it is necessary to determine the exact labeling density of your sample, see instructions in *Label* IT® Frequently Asked Questions or Tips from the Bench.

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▸ *Label* IT® TRACKER™ LABELING APPLICATIONS

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Table 1: *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kits and excitation/emission wavelengths:

Label IT Product Name	Excitation Wave-length (nm)	Emission Wave-length (nm)	Product No.
Label IT® Tracker™ Intracellular Nucleic Acid Localization Kit, Cy®3	550	570	MIR 7020
Label IT® Tracker™ Intracellular Nucleic Acid Localization Kit, Cy®5	649	670	MIR 7021
Label IT® Tracker™ Intracellular Nucleic Acid Localization Kit, CX-Rhodamine	576	597	MIR 7022
Label IT® Tracker™ Intracellular Nucleic Acid Localization Kit, TM-Rhodamine	546	576	MIR 7023
Label IT® Tracker™ Intracellular Nucleic Acid Localization Kit, Biotin	n/a	n/a	MIR 7024
Label IT® Tracker™ Intracellular Nucleic Acid Localization Kit, Fluorescein	492	518	MIR 7025

A. *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.

B. *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, 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. Biotin Detection

The tracking of Biotin-labeled DNA allows the use of a wide variety of commercially available detection reagents. Furthermore, the potential for multi-color tracking experiments is enhanced when the experimental design includes detection of a Biotin-labeled plasmid with a unique fluorescent conjugate and the direct detection of Cy®3 , Cy®5, Rhodamine or Fluorescein-labeled plasmid(s). For the recommended post-Labeling avidin/streptavidin conjugation procedure, see the Full Protocol.

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Mirus Bio LLC
www.mirusbio.com | techsupport@mirusbio.com | U.S. Toll Free: 844.MIRUSBIO (844.647.8724) | Direct: +1.608.441.2852