Label IT® siRNA Tracker Intracellular Localization Kits

Quick Reference Protocol

Instructions for MIR 7212, 7213, 7214, 7215, 7216, 7217

Full protocol, SDS and Certificate of Analysis available at mirusbio.com/7212

SPECIFICATIONS

Storage	Store *Label* IT® siRNA Tracker Reagent at –20°C in both dried and reconstituted form. Store Reconstitution Solution, 10X Labeling Buffer A, and siRNA Dilution Buffer at –20°C.
Product Guarantee	The *Label* IT® siRNA Tracker 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® siRNA TRACKER LABELING REACTION

Full protocol and additional documentation available at ***mirusbio.com/7212***

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

1. Warm the *Label* IT® siRNA Tracker Intracellular Localization Kit to room temperature.
2. Briefly centrifuge the *Label* IT® siRNA Tracker Reagent to collect the pellet.
3. For first use only, add 50 µl of Reconstitution Solution to the *Label* IT® Reagent pellet and mix well.

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

Labeling Reaction Example for siRNA Duplexes:	
Molecular Biology-grade H_20	60 µl
10X Labeling Buffer A	10 µl
siRNA duplex*	20 µl (40 µM stock)
Label IT® siRNA Tracker Reagent	10 µl
Total Volume:	**100 µl**

***NOTE:** This example labels ~10 µg siRNA duplex at a 1:1 (v:w) ratio of Label IT® Reagent to siRNA for a labeling density of 1 label per 15-40 bases. To modify the labeling density, increase or decrease the amount of Label IT® Reagent in the reaction or adjust the incubation time. The Label IT® Reagent should never exceed 20% of the total reaction volume.*
If using **single stranded siRNA, add 40 µl siRNA (40 µM stock) and reduce the Molecular Biology-grade H_20 to 40 µl in the reaction to maintain the 1:1 (v:w) ratio.*

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® siRNA Tracker Reagent by ethanol precipitating labeled siRNA.

1. Add 0.1 volume of 5 M sodium chloride and 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 siRNA. Gently remove the ethanol with a micropipetter; do not disturb the pellet.
NOTE: Mark and orient the precipitate-containing tubes in the microfuge such that the pellet will form in a predetermined place as small nucleic acid quantities can be difficult to visualize.
3. Wash the pellet once with 500 µl room temperature 70% ethanol. Centrifuge at full speed in a refrigerated microcentrifuge for an additional 15-30 minutes.
4. Remove all traces of ethanol with a micropipetter. DO NOT allow the sample to air dry for longer than 5 minutes as the pellet may become difficult to resuspend.
5. Resuspend labeled siRNA in an appropriate volume of siRNA Dilution Buffer. If following the above example, add 20 µl (40 µl for single stranded siRNA) for an approximate final concentration of 40 µM.
6. If an exact concentration is required, quantify the purified, labeled siRNA on a spectrophotometer and dilute to the desired working condition (e.g. 10 µM).
7. Store the purified, labeled siRNA 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 15-40 bases can be expected if using a 1:1 (v:w) ratio of *Label* IT® siRNA Tracker Reagent to siRNA 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® siRNA TRACKER LABELING APPLICATIONS

Full protocol and additional documentation available at ***mirusbio.com/7212***

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

Label IT Product Name	Excitation Wavelength (nm)	Emission Wavelength (nm)	Product No.
Label IT® siRNA Tracker Intracellular Localization Kit, Cy®3	550	570	MIR 7212
Label IT® siRNA Tracker Intracellular Localization Kit, Cy®5	649	670	MIR 7213
Label IT® siRNA Tracker Intracellular Localization Kit, CX-Rhodamine	576	597	MIR 7214
Label IT® siRNA Tracker Intracellular Localization Kit, TM-Rhodamine	546	576	MIR 7215
Label IT® siRNA Tracker Intracellular Localization Kit, Fluorescein	492	518	MIR 7216
Label IT® siRNA Tracker Intracellular Localization Kit, Biotin	n/a	n/a	MIR 7217

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. For instructions on detection of *Label* IT® siRNA Tracker fluorescently labeled siRNA on mounted coverslips (fixed cells), see the Full Protocol.

B. *In Vivo* Tracking Experiments

Subcellular localization and reporter transgene expression can be monitored following the introduction of labeled siRNA into mammalian cells *in vivo*. The *Trans*IT®-QR Hydrodynamic Delivery Solution is designed specifically for the safe and efficient delivery of plasmid DNA and siRNA into laboratory mice using the hydrodynamic tail vein injection procedure. Nucleic acids delivered with this kit primarily target the liver, with lower levels of expression detected in the spleen, lung, heart and kidneys.

C. Biotin Detection

A variety of commercially available secondary detection fluorescent conjugates are compatible with siRNA labeled using the *Label* IT® siRNA Tracker Biotin Reagent. Furthermore, the potential for multi-color tracking experiments is enhanced when the experimental design includes detection of a Biotin-labeled siRNA with a fluorophore conjugate and the direct detection of Cy®3 , Cy®5, Fluorescein or Rhodamine-labeled siRNA(s). For instructions on detection of *Label* IT® siRNA Tracker Biotin labeled siRNA on mounted coverslips (fixed cells), see the Full Protocol.

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