Frequently Asked Questions

Label IT® Nucleic Acid Labeling Kits

INTENDED USE

The *Label* IT® Nucleic Acid Labeling Kits designed by the nucleic acid specialists of Mirus Bio Corporation offer efficient, one-step, direct labeling for any type of nucleic acid. This non-enzymatic labeling method covalently attaches labels to nucleic acids in a non-destructive manner. The *Label* IT® Kits are available in a variety of labels including Cy™3, Cy™5, CX-Rhodamine, TM-Rhodamine, Fluorescein, Digoxin, Biotin, and Dinitrophenyl (DNP). *Label* IT® Nucleic Acid Modifying Kits are also available for the modification of DNA or RNA with amine or carboxylic acid functional groups.

In addition to the general *Label* IT® Nucleic Acid Labeling Kits, optimized labeling kits are available for the following applications:

- *In Vitro* and *In Vivo* tracking of plasmid DNA (*Label* IT® Tracker™ Kits)
- *In Vitro* and *In Vivo* tracking of siRNA (*Label* IT® siRNA Tracker™ Kits)
- Microarrays (*Label* IT® uArray™ Labeling Kits)
- Flourescence *In Situ* Hybridization (*Label* IT® FISH Kits)
- Southern blots, dot blots and colony lifts (HybQUEST® Kits)

The general *Label* IT® Kits are featured in this FAQ. For questions specific for the other kits, please visit our website at www.mirusbio.com or call Technical Support at 888.530.0801.

FREQUENTLY ASKED QUESTIONS

Q1. How do the *Label* IT® Reagents label nucleic acids?

The *Label* IT Labeling Reagent comprises a reactive alkylating agent with strong nucleic acid binding capability facilitated via electrostatic interactions. Labeling via covalent modification (alkylation) of RNA or DNA can take place on reactive heteroatoms on any nucleotide of the nucleic acid polymer. The label does not impact hybridization performance in subsequent microarray applications.

Q2. What types of nucleic acids can I label with the *Label* IT® Reagents?

Any nucleic acid can be labeled using the *Label* IT® Reagents.

Q3. What labeling efficiencies can I expect if I follow the *Label* IT® Reagent protocol?

We routinely achieve one label every 20-60 base pairs using the standard labeling protocol and a spectrophotometric assay to estimate the density of labels. See Q26 for details about the calculations.
Note: Labeling densities may be different if you are using an application-specific labeling kit.

Q4. What are the size limits of the nucleic acid that can be labeled with the *Label* IT® Reagents?

Any purified nucleic acid which contains guanine residues should be successfully labeled with our *Label* IT® Reagents, using the conditions described in the protocol. We routinely label nucleic acids as short as 20 nucleotides and as long as many kilobases.

Q5. How should I store the *Label* IT® Reagents and the labeled nucleic acid?

Store dried and reconstituted *Label* IT® Reagents and labeled nucleic acids tightly capped at -20°C. Protect the *Label* IT® Reagents from exposure to light and moisture. Fluorescently labeled nucleic acids must also be stored protected from light. Improper storage may result in decreased labeling efficiencies.

Q6. Can I label dNTPs with the *Label* IT® Reagents?

We do not recommend the use of the *Label* IT® Reagents to modify dNTPs. The smallest substrates for successful *Label* IT® reactions are believed to be short oligos with a minimum of 3 negative charges.

Q7. Can I scale the labeling reaction up or down?

Yes. It is possible to change the labeling density, the volume of the reaction and the amount of nucleic acid that is labeled. To modify the labeling density, simply change the ratio of the labeling reagent to nucleic acid during the labeling reaction or adjust the incubation time of the labeling reaction. In addition, the volume of the labeling reaction may be scaled up or down, simply use an amount of *Label* IT® Reagent that does not consist of more than 20% of the total reaction volume and the Labeling Buffer A diluted to 1X final concentration in the reaction.

Q8. How will increased incubation times affect the labeling efficiency?

When using the general *Label* IT® Nucleic Acid Labeling Kits, we observe a linear increase in labeling efficiency during the first 3 hours of incubation at 37°C. We recommend a standard reaction for 1 hour at 37°C. However, due to the linear nature of the labeling reaction, you can use twice as much *Label* IT® Reagent and incubate for 30 minutes at 37°C and achieve similar labeling efficiency.

Note: Labeling densities may be different if you are using an application-specific labeling kit (listed in the bulleted points above). These more specific kits have been optimized to achieve the optimal labeling density for the specific application with a 1 hour labeling reaction at 37°C.

Q9. How will increasing the amount of *Label* IT® Reagent (per 1 ug nucleic acid) affect the labeling efficiency?

Generally, modest changes (2-4 fold) in the ratio of *Label* IT® Reagent to nucleic acid affect the labeling efficiency in a linear manner. Dramatic increases to the recommended 1:1 (µl *Label* IT® Reagent : µg DNA) ratio will result in increased labeling, but not necessarily of a linear nature. Dramatic increases to the recommended labeling ratio may also increase the chance of nicking the nucleic acid template.

Q10. Once I have labeled my sample, how can I avoid cross-labeling any other DNA that I subsequently add to my sample?

The covalent bonds between the label and the nucleic acids bases are very stable and will not allow cross labeling or transfer of labels to other species.

Q11. Why should labeled DNA probes be denatured with Mirus' Denaturation Reagent D1 (included in kit)?

This alkaline denaturation process stabilizes the labeled DNA product and has shown increased hybridization sensitivity when compared to heat denaturation alone. If necessary, heat denaturation can be performed after an initial D1/N1 treatment.

Q12. Can labeled RNA be denatured with Mirus Denaturation Reagent D1?

No. Alkaline conditions destroy RNA. RNA should be denatured by heating at 65°C for 10 minutes.

Q13. Will dithiothreitol (DTT) or RNase inhibitors have an effect on the labeling reaction?

DTT will have no effect on the labeling reaction. Any proteinacious RNase inhibitors that are added before the labeling reaction will compete modestly with the nucleic acid sample for the *Label* IT® Reagent, as the *Label* IT® Reagents have a greater affinity for nucleic acids. However, if the RNase inhibitors are added after the labeling is performed, they will have no effect on the product.

Q14. Can "dirty" DNA affect the labeling reaction?

Yes. For best results, use clean, intact nucleic acid in labeling reactions.

Q15. Can labeled DNA be transfected?

Yes. Mirus Bio has successfully delivered fluorescently labeled (and non-fluorescently labeled with subsequent detection) DNA into a variety of cell lines using various transfection reagents for DNA transport and gene expression studies. Mirus Bio has developed the *Label* IT® Tracker Intracellular Nucleic Acid Localization Kits (see Related Products section) for this purpose. However, it is important to note that if the nucleic acid to be transfected is highly labeled, transcription and translation will likely be inhibited. This will result in significantly less transgene expression than is observed with transfections using unlabeled control nucleic acid.

Q16. Can labeled siRNA be transfected?

Yes. Mirus Bio has successfully delivered fluorescently labeled siRNA into a variety of cell lines using either *Trans*IT-TKO® or *Trans*IT®-siQUEST™ for siRNA tracking and gene suppression studies. Mirus Bio has developed the *Label* IT® siRNA Tracker Kits (see Related Products section) for this purpose. Please note that the *Label* IT® siRNA Tracker Kits should not interfere with RNAi knockdown.

Q17. Can labeled DNA be used for DNA-binding studies such as gel-shift assays?

Since the *Label* IT® Reagent covalently modifies nucleic acid residues, it may interfere with the binding of some proteins. The ratio of *Label* IT® Reagent to DNA can be modified during the labeling reaction to allow for fewer labeled residues per DNA molecule; however, the DNA will have to be tested in each specific binding application to determine biological activity.

Q18. Can I directly visualize nucleic acids labeled with Digoxin, DNP or Biotin?

On an ethidium bromide-stained agarose gel, you may see a slower migration rate with labeled DNA compared to unlabeled control DNA. Alternatively, detection can be achieved using suitable antibody or avidin conjugates and substrates for dot blot applications. Mirus Bio has successfully used conjugated antibodies/avidins from a variety of commercial sources.

Q19. Can I directly visualize nucleic acids labeled with CX-Rhodamine, TM-Rhodamine, Fluorescein, Cy™3, or Cy™5 on a gel?

Yes. Labeled nucleic acid can be analyzed by agarose gel electrophoresis (without ethidium bromide staining) and then visualized on a UV transilluminator. The bands will appear faint because UV bulbs in the transilluminator emit light at approximately 300 nm (for a standard transilluminator with "midrange" bulbs). This is not the optimal excitation wavelength for the fluorescent labels.

Q20. Can I use the *Label* IT® Reagents for traditional hybridization applications?

Yes. The *Label* IT® Reagents are ideal for the preparation of non-radioactively labeled probes for hybridization applications. The preparation of Biotin, Digoxin, DNP and Fluorescein labeled DNA probes have been optimized in our HybQUEST® *Label* IT® Kits and can be easily substituted in your standard hybridization and detection protocol. The preparation, hybridization, and detection of DNP-labeled DNA probes for membrane-based hybridization applications has been optimized in our complete HybQUEST® Kit. LOAD'n GLO Markers are also available for non-radioactive Southern blot detection (see Related Products section).

Q21. Can I use the *Label* IT® Reagents for FISH applications?

The *Label* IT® FISH Kits (see Related Products section) are ideal for the preparation of probes for FISH (probing of specific DNA sequences on chromosomes in metaphase spreads and/or interphase nuclei) applications. Depending on the quantity of repetitive sequences in the chosen FISH probe, the addition of blocking DNA may be necessary to reduce non-specific background signal. The most common suppressor/blocker DNAs are species-specific cot-1 DNA and sheared salmon sperm DNA.

Q22. Can proteins be labeled?

Yes. The *Label* IT® Reagents can label primary amines that are present on proteins. However, the *Label* IT® Reagents have a higher affinity for nucleic acids than for proteins and therefore label proteins rather inefficiently. On the other hand, if there is a high concentration of protein or *Label* IT® Reagent in the reaction, proteins can be labeled.

Q23. Can *Label* IT® Reagents be used for oligonucleotide labeling?

Yes. Oligonucleotides can be labeled just as efficiently as larger DNA or RNA molecules. To achieve efficient labeling of short oligos with the general *Label* IT® Reagents, we recommend increasing the ratio of the *Label* IT® Reagent to nucleic acid by at least 2 fold or extending incubation time to 2 hours or more. For labeling siRNA oligos, for cellular tracking applications, Mirus Bio Corporation also offers the *Label* IT® siRNA Tracker Kits (see Related Products section).

Q24. What are the excitation and emission wavelengths for CX-Rhodamine, TM-Rhodamine, Fluorescein, Cy™3 and Cy™5 dyes?

Labeling Reagent	Excitation Wavelength (nm)	Emission Wavelength (nm)
5-carboxy-X-rhodamine	576	597
Tetramethyl rhodamine	546	576
5-carboxy fluorescein	492	518
Cy™3	550	570
Cy™5	649	670

Q25. Can I use the *Label* IT® Reagents for microarray applications?

The *Label* IT® μArray Labeling Kits (see Related Products section) are specifically optimized for labeling microarray samples. Microarray applications require a high labeling density for optimal signal generation. Mirus Bio offers three different *Label* IT® μArray Labeling Kits:

Catalog Number	*Label* IT® μArray Labeling Kit	*Label* IT® Reagents Included	Microarray platform	Notes
MIR 8010, 8050	Biotin	Biotin	Single channel *(one sample per array)*	Requires detection reagent(s) *(not included)*
MIR 8105, 8125	Dual	Biotin & Fluorescein	Dual channel *(two samples per array)*	Requires detection reagents *(not included)*
MIR 8205, 8225	Cy™3/Cy™5	Cy™3 & Cy™5	Dual channel *(two samples per array)*	No additional detection reagents required

Each of these kits comes with a protocol for labeling mRNA, cDNA, or cRNA samples, depending on the material that will be hybridized to the microarray.

One advantage of the use of *Label* IT® reagents for microarray samples is the ability to directly label and hybridize RNA samples, eliminating enzymatic replication and incorporation steps. Direct mRNA labeling is compatible with cDNA arrays and *anti-sense strand* oligo arrays. Alternate sample types (e.g. ribosomal RNA, bacterial RNA, microRNA, genomic DNA) can also be labeled with the *Label* IT® μArray Labeling Kits.

Q26. Can I use the *Label* IT® Reagents to attach my DNA to a glass slide or bead?

The *Label* IT® Modifying Reagents (see Related Products section), which directly modify nucleic acids with amine or carboxylic acid functional groups, are designed for custom dye or solid surface attachment chemistry. For example, amine modified nucleic acids can be attached to silylated (aldehyde) slides or beads and carboxylic acid nucleic acids can be attached to hydrazide coated plates and microspheres.

Q27. How do I know if my nucleic acid is labeled?

A. Fluorescent labels (Cy™3, Cy™5, TM-Rhodamine, CX-Rhodamine, Fluorescein)

The relative density of fluorescent labels on purified, labeled nucleic acid can be assessed by:

1. **Agarose gel electrophoresis without ethidium bromide.** The nucleic acid will appear faint under UV illumination because the transilluminator emits at approximately 300 nm, which is not optimal for the fluorescent labels (see Table 1). With subsequent ethidium bromide staining, a gel shift may be detected (relative to unlabeled nucleic acid), indicating labeling of the nucleic acid.
2. **Spectrophotometric absorbance at λ_{max}.** Several µg of labeled nucleic acid may be required to generate significant λ_{max} absorbance readings.
3. **Fluorimetric detection at the specific excitation and emission wavelengths**
4. **Fluorescent microscopy.** Spot dilutions of labeled nucleic acid onto a glass slide and view with fluorescent microscope.
5. ***In vitro* tracking.** Transfect labeled DNA into a common cell line, such as COS-7, HeLa, or NIH 3T3 to ascertain detection efficiency and optimal parameters.

B. Epitopes (Digoxin, Biotin, DNP, Fluorescein)

1. **Gel shift.** With ethidium bromide staining, a gel shift may be detected (relative to unlabeled DNA), indicating labeling of the nucleic acid.
2. **Dot blots.** Labeled nucleic acid can be spotted on a nylon membrane and detected with epitope-specific antibody or streptavidin conjugates.

Q28. What is the recommended protocol for estimating the number of fluorescent labels per nucleic acid molecule?

The most straight-forward way to estimate the number of fluorescent labels on the DNA (or RNA) molecule involves measuring the absorbance of the nucleic-dye conjugate at 260 nm (A_{260}) and the λ_{MAX} for the particular dye (A_{dye}). For most applications, the absorbance of the entire sample, using a spectrophotometer with a microcell, may be required to generate reliable absorbance readings. If samples are going to be quantified using spectrophotometry, we recommend purifying the labeled nucleic acid, by ethanol precipitation or with a silica membrane-based column. While gel filtration is an effective purification method, we observe erroneously high spectrophotometer readings (at A_{260}) when using this method. To quantify your labeled sample, choose from 1 of the 2 following methods:

Calculate Base: Dye Ratio

1. Correct for the contribution of the dye to the A_{260} reading using:

$A_{base} = A_{260} - (A_{dye} * CF_{260})$

2. Calculate the ratio of bases to dye molecules:

base:dye = $(A_{base} * \varepsilon_{dye}) / (A_{dye} * \varepsilon_{base})$

Calculate pmol of dye per µg of nucleic acid:

- dye concentration, mol/l = $A_{dye}/\varepsilon_{dye}$
- pmol dye in sample = mol/l dye * 10^{12} pmol/mol * sample volume (l)
- µg DNA = A_{base} * 50 µg/ml (for ds DNA) * sample volume (ml)
  (Use 40 µg/ml for RNA)
- divide pmol dye/ µg DNA in sample

Dye	(ε_{dye}) Extinction Coefficient of Nucleic Acid Bound Dye (M^{-1} cm^{-1})	C.F. $_{260}$	λmax (nm)

Cy™3	150,000	0.08	550
Cy™5	250,000	0.05	649
Fluorescein	30,000	0.32	494
TM-Rhodamine	100,000	0.27	546
CX-Rhodamine	82,000	Not determined	576
Nucleic Acid	**(ε_{base}) Extinction Coefficient of Nucleic Acid (M^{-1} cm^{-1})**		
ds DNA	6,600		
ss DNA	8,919		
oligonucleotide	10,000		
RNA	8,250		

Related Products

For tracking studies:

DNA Tracking:

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

RNAi Tracking:

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

Label IT®siRNA Tracker Intracellular Localization Kit with *Trans*IT®- 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® µArray® Biotin Labeling Kits (Product # MIR 8010 and MIR 8050)

Label IT® µArray® Dual Labeling Kits (Product # MIR 8105 and MIR 8125)

Label IT® µArray® Cy™3/ Cy™5 Labeling Kits (Product # MIR 8105 and MIR 8125)

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)

For Nucleic Acid Modification:

Label IT® Amine Modifying Kits (Product # MIR3900, 3925)

Label IT® Amine Modifying Kits (Product # MIR4000, 4025)

*These products are available in additional sizes; please see www.mirusbio.com.

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Mirus Transfection 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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