

# Label IT® Nucleic Acid Labeling Kits

## Quick Reference Protocol



Instructions for MIR 3100, 3125, 3200, 3225, 3400, 3425, 3600, 3625, 3700, 3725, 3900, 3925, 7100, 7125.

Full protocol, SDS and Certificate of Analysis available at [mirusbio.com/literature](http://mirusbio.com/literature)

<b>Storage</b>	Store <i>Label IT</i> ® Reagent, Reconstitution Solution and 10X Labeling Buffer A at -20°C. Store G50 spin columns at 4°C. Do <b>not</b> use if frozen.
<b>Product Guarantee</b>	Lyophilized <i>Label IT</i> ® Reagent and other reagents are stable for 1 year from the date of purchase, when properly stored and handled. Reconstituted <i>Label IT</i> ® Reagent is stable at -20°C for 6 months.
<b>Kit Sizes and Usage</b>	25 µg size kits contain sufficient reagents to label 25 µg of nucleic acid; 100 µg size kits contain sufficient reagents to label 100 µg nucleic acid.

### ▶ TO LABEL NUCLEIC ACIDS



Full protocol and additional documentation available at [mirusbio.com/literature](http://mirusbio.com/literature)

## Reconstitute *Label IT*® Reagent & Prepare Labeling Reaction

1. Warm the *Label IT*® kit components to room temperature before use and briefly centrifuge the *Label IT*® Reagent to collect the lyophilized pellet.
2. Reconstitute the pellet with the appropriate volume of Reconstitution Solution and mix well.
  - Add 25 µl for a 25 µg size kit.
  - Add 100 µl for a 100 µg size kit.
3. Prepare the labeling reaction according to the table below in a sterile tube. Add the *Label IT*® Reagent **last**.

For most applications, a 1:1 ratio will be suitable, however more sensitive applications will require lower labeling at a 0.5:1 ratio. See [full protocol](#).

### *Label IT*® Reaction

Ratio of <i>Label IT</i> ® Reagent : Nucleic Acid (µl:µg)	1:1	0.5:1
DNase & RNase-free water	35 µl	37.5 µl
10X Labeling Buffer A	5 µl	5 µl
1 µg/µl Nucleic Acid Sample	5 µl	5 µl
<i>Label IT</i> ® Reagent (add last)	5 µl	2.5 µl
<b>Total Reaction Volume</b>	<b>50 µl</b>	<b>50 µl</b>

### ▶ Labeling Density

Using a 1:1 ratio will yield approximately 1 label every 20-60 base pairs. This is optimal for most applications, but more sensitive applications may require a lower density, which can be achieved with a 0.5:1 ratio.

4. Incubate at 37°C for 1 hour.
5. Purify the labeled nucleic acid using G50 spin columns (alternatively use ethanol precipitation method - see page 2.)
  - a. Vortex the column briefly to resuspend the resin, loosen the cap by a quarter-turn and twist off the bottom closure.
  - b. Place the column in a 1.5 ml tube and centrifuge for 1 minute at 735xg. Discard the effluent and repeat once.
  - c. Move the column to a new sterile tube. Slowly apply the 50 µl nucleic acid sample to the column without disturbing the resin bed.  
*Use exactly 50 µl per column. If lower, make up to volume with 1X Labeling Buffer A. If higher, use 50 µl per column, i.e. use 3 columns for 150 µl.*
  - d. Centrifuge for 2 minutes at 735xg. The purified nucleic acid will collect in the tube.
6. Store the labeled sample at -20°C protected from light.

## For Research Use Only

Mirus Bio LLC

www.mirusbio.com | techsupport@mirusbio.com | U.S. Toll Free: +1.844.647.8724 | Direct: +1.608.441.2852

# Label IT® Nucleic Acid Labeling Kits

## Quick Reference Protocol



Instructions for MIR 3100, 3125, 3200, 3225, 3400, 3425, 3600, 3625, 3700, 3725, 7100, 7125, 3900, 3925.

Full protocol, SDS and Certificate of Analysis available at [mirusbio.com/literature](https://mirusbio.com/literature)

### Alternate Purification Method : Ethanol Precipitation

If the *Label IT*® reaction volume is less than 100 µl, make up to volume with 1X Labeling Buffer A or nuclease free water before proceeding.

1. Add 0.1X volume (i.e. 10 µl in 100 µl total volume) of 5M sodium chloride and 2X volume of -20°C pre-chilled 100% ethanol to the labeled nucleic acid. Mix well and incubate for 20 minutes at -20°C.
2. Centrifuge at full speed (>14,000xg) for 30 minutes at 4°C to pellet the nucleic acid.
3. Carefully discard the supernatant without disturbing the pellet.
4. Add 500µl room temperature 70% ethanol and centrifuge again at full speed for 15 minutes.
5. Remove all traces of ethanol supernatant without disturbing the pellet. Do not allow the pellet to over-dry (i.e. not longer than 5 minutes unless there are visible ethanol traces).
6. Add desired volume of 1X Labeling Buffer A or nuclease-free water and allow to redissolve for 5-30 minutes, followed by gentle pipetting to mix.
7. Store the labeled nucleic acid at -20°C, protected from light.

### Label IT® Configurations and Tips

Label IT® Configuration	Excitation/Emission (nm)	Catalog No. (25µg / 100µg size)
Label IT® Fluorescein	492/518	MIR 3225 / MIR 3200
Label IT® MFP488	501/523	MIR 7125 / MIR 7100
Label IT® Cy™3	550/570	MIR 3625 / MIR 3600
Label IT® CX-Rhodamine	576/597	MIR 3125 / MIR 3100
Label IT® Cy™5	649/670	MIR 3725 / MIR 3700
Label IT® Amine	-	MIR 3925 / MIR 3900
Label IT® Biotin	-	MIR 3425 / MIR 3400

#### ► Calculating Labeling Density

Instructions for calculating the labeling density can be found at [mirusbio.com/calculate-nucleic-acid-labeling-density](https://mirusbio.com/calculate-nucleic-acid-labeling-density). The relative labeling density can be estimated by dot blot and gel shift analysis.

#### ► Adjusting Labeling Density

Adjusting the labeling density can be achieved by altering the ratio of *Label IT*® Reagent to nucleic acid and/or altering the incubation time at 37°C. Within limits, the relationship between incubation time and labeling density is linear i.e. to achieve half the labeling density, reduce the incubation time by half. Refer to [full protocol](#) or contact [techsupport@mirusbio.com](mailto:techsupport@mirusbio.com).