

***Label IT*[®] miRNA Labeling Kits**

INTENDED USE

Microarrays represent an established genomics technology that allows the simultaneous hybridization of multiple target molecules on a solid support (i.e. glass slide). Expression profiling analysis, a prominent microarray application, measures the presence and relative amount of specific RNA transcripts by quantifying fluorescent signal from the microarray hybridization. For expression profiling microarray applications, samples must be highly labeled with a marker molecule for detection. The *Label IT* Cy[™] 3 and Cy[™] 5 miRNA Reagents allow one-step, direct labeling of samples (containing miRNAs) with no enzymatic steps or alterations to sequence or length. The *Label IT* miRNA Labeling Kits provides the optimal labeling density for microarray applications using the popular Cy[™] 3 and/or Cy[™] 5 fluorophores, characterized by high fluorescent signal with minimal quenching. These fluorophores can be detected by standard microarray scanners with no additional detection reagents or steps required. Following purification, labeled samples are ready for hybridization.

FREQUENTLY ASKED QUESTIONS

Q1. What are microRNAs (miRNAs)?

miRNAs are endogenous, small (~22 nt) regulatory RNAs that participate in natural RNA interference (RNAi) in plants and animals. miRNAs regulate gene expression during differentiation and development through post-transcriptional effects on target mRNA stability and translational efficiency.

Q2. How are miRNA expression patterns determined?

Techniques for miRNA expression profiling are evolving and existing techniques include northern blotting, cloning efficiency, qRT-PCR, and microarray analysis.

Q3. How are miRNAs analyzed by microarray?

Microarrays allow the expression profiling of many miRNAs in a single experiment. An array surface (glass, membrane) is spotted with capture DNA oligos complementary to mature miRNA sequences. Purified small RNA samples, enriched for miRNAs, are labeled and hybridized to spotted oligo arrays to determine a miRNA expression profile. The *Label IT* miRNA Labeling Kits are used to label miRNA samples with Cy[™] 3 or Cy[™] 5 for microarray analysis.

Q4. How do the *Label IT* miRNA Kit label miRNAs?

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 (including miRNA) 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.

Q5. Can I scale the labeling volume up or down?

Yes. The volume of the labeling reaction may be scaled up or down, depending on the amount/volume of nucleic acid to be labeled. We recommend that a minimum reaction volume be used such that the amount of *Label IT* Reagent constitutes less than 20% of the total reaction volume and the Labeling Buffer M is diluted to 1X final concentration in the reaction.

Q6. How will increased incubation times affect the labeling efficiency?

Labeling density increases during the first 3 hours of incubation at 37°C. Longer incubation times may increase the chance of nicking the nucleic acid template. We recommend a standard reaction for 1 hour at 37°C. Increased labeling density may be achieved by titrating the amount of *Label IT* Reagent in the reaction.

Q7. 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 acid bases are very stable and will not allow cross labeling or transfer of labels to other species. Use the 10X STOP Solution to terminate the labeling reaction. Unreacted dye is removed during purification.

Q8. Can poorly enriched RNA affect the hybridization?

Yes. For best results, ensure enrichment procedure resulted in RNA species with the size range of < 200 nucleotides. Follow isolation protocol recommendations to ensure quality enrichment.

Q9. How should I store the *Label IT*[®] Reagents and the labeled miRNA enriched sample?

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.

Q10. How do I know if my nucleic acid is labeled?

The relative density of fluorescent labels on purified, labeled miRNA enriched samples is assessed by:

1. Acrylamide gel electrophoresis without nucleic acid staining. The miRNA enriched sample will appear faint under UV illumination because the transilluminator emits at approximately 300 nm, which is not optimal for the fluorescent labels.

2. Spectrophotometric absorbance at λ_{max} . Microgram amounts of labeled miRNA enriched samples may be required to generate significant λ_{max} absorbance readings. See Q11 for details.

3. Fluorimetric detection at the specific excitation and emission wavelengths

4. Fluorescent microscopy. Spot dilutions of labeled miRNA enriched samples onto a glass slide and view with fluorescent microscope.

Q11. What is the recommended protocol for estimating the number of labels per RNA molecule?

The most straight-forward way to estimate the number of fluorescent labels on the RNA molecule involves measuring the absorbance of the labeled sample at 260 nm (A_{260}) and the λ_{dye} for the particular dye. For most applications, the absorbance of the entire sample, using a spectrophotometer with a microcell, may be required to generate reliable absorbance readings.

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

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

Calculate the ratio of bases to dye molecules:

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

Another way to interpret the same data is to calculate pmol of dye per μ g of recovered nucleic acid:

- dye concentration, mol/l = A_{dye} / ϵ_{dye}
- pmol dye in sample = mol/l dye * 10^{12} pmol/mol * sample volume (l)
- μ g miRNA enriched RNA = $A_{260} * 33 \mu\text{g/ml} * \text{sample volume (ml)}$

Dye	(ϵ_{dye}) Extinction Coefficient of Nucleic Acid Bound Dye ($M^{-1} cm^{-1}$)	C.F. 260	λ_{max} (nm)
Cy [™] 3	150,000	0.08	550
Cy [™] 5	250,000	0.05	649
Nucleic Acid	(ϵ_{base}) Extinction Coefficient of Nucleic Acid ($M^{-1} cm^{-1}$)		
Single strand RNA (21 mer)	9,700		