MISS NOTHING

*Label IT®* miRNA Labeling Kit accurately detects all microRNAs in a sample including those our competitors systematically miss.
As Close to Native as You Can Get

### Chemical Labeling Methods
- **Direct chemical labeling with Label IT**
- **Reverse transcription (RT)** with labeled random primers
- **RT plus 3’ end tailing with labeled nucleotides**
- **Adaptor ligation, RT, PCR with labeled nucleotides**
- **T7 adaptor ligation, RT, PCR, T7 transcription with labeled nucleotides**
- **3’ end labeling with poly(A) polymerase**
- **Ligation of labeled di-nucleotides**

### Enzymatic Labeling Methods
- **Labeling using the Label IT method** maintains the integrity of the original miRNA species. Exogenous nucleotides added by enzymatic labeling or truncated reverse transcription (RT) products may decrease hybridization performance and increased non-specific hybridization.

**Figure 1.** It is possible to label miRNA samples using a variety of chemical and enzymatic methods. Direct chemical labeling using the Label IT method maintains the integrity of the original miRNA species. Exogenous nucleotides added by enzymatic labeling or truncated reverse transcription (RT) products may decrease hybridization performance and increased non-specific hybridization.

**Figure 2.** The Label IT method is composed of a fluorophore attached to the positively charged Label IT linker and reactive alkylating group. The Label IT reagent covalently attaches fluorophores to any reactive heteroatom in the miRNAs without affecting downstream hybridization performance. miRNA labeling and purification can be completed in only two hours.

**Figure 3.** Discrepant microarray miRNA profiles are obtained from chemical and enzymatic labeling methods. miRNA-enriched mouse heart and brain samples were hybridized to miRNA microarrays after labeling with either Label IT or microRNA Labeling Kit. Positive M values, expressed as the log transformed ratio of heart/brain signal, represent mouse miRNAs preferentially expressed in heart tissue, while negative values correspond to miRNAs preferentially expressed in brain. Only the miRNA expression profile generated using the Label IT method corroborates the established expression pattern obtained by northern blotting.  


### Accurately Detect All miRNAs in Your Sample

**Figure 4.** Synthetic RNA oligonucleotides representing control and test miRNAs were spiked into miRNA-enriched samples before chemical or enzymatic labeling and hybridization (A). Synthetic control miRNAs were consistently detected on microarrays using all three labeling methods. Synthetic test miRNAs exhibited different profiles depending on the labeling method used. In each case, 10 fmol of each RNA oligonucleotide was spiked to the sample that did not contain the specific miRNA endogenously. Accordingly, miR-219 (test) and miR-124a (control) were each spiked into heart RNA, and miR-208 (test) and miR-1 (control) were each spiked into brain RNA. Results are presented (B).

**Specific miRNAs are Not Detected by Enzymatic Labeling Methods**
miRNA EXPRESSION PROFILING ▶ Universal Labeling

**Sequence-independent Labeling**

![Graph showing sequence-independent labeling](image)

**Source-independent Chemical Labeling of RNA**

![Diagram showing direct chemical labeling and enzymatic labeling](image)

Figure 5. Synthetic RNA oligonucleotides representing miRNAs with no Gs (miR-467a), no As (miR-328), no Cs (miR-206), and no Us (miR-214) were chemically labeled in triplicate with the Label IT® Cy™3 reagent, purified and spectrophotometrically measured to estimate labeling density (pmol Cy™3/µg RNA). Average labeling densities are plotted. Similar results were observed with Cy™5 labeling reactions (data not shown).

Figure 6. (A) Mammalian miRNAs can be labeled using direct chemical labeling (Label IT® miRNA Kits) or commercially available enzymatic labeling methods (miVana™ and NCode™). The poly(A) polymerase-based enzymatic methods generate long 3' tails which dramatically extend the length of the mammalian miRNA species, whereas the Label IT® technology adds only the labels internally. (B) Endogenous methylation of plant miRNAs at their 3' ends inhibits labeling by the poly(A) polymerase-based kits. In contrast, plant miRNAs are efficiently labeled using the Label IT® Kits (Figure 8) due to its direct chemical labeling technology.

Detect Subfemtomolar miRNAs

Figure 7. A synthetic RNA oligonucleotide equivalent to miR-124a miRNA was labeled with either Cy™3 or Cy™5 using the Label IT® miRNA Labeling Kit. Defined quantities (0, 0.08, 0.4, and 2 fmol) of each labeled miRNA were spiked into aliquots of pooled Cy™3 and Cy™5 labeled heart miRNA-enriched small RNA samples, which do not contain brain-specific miR-124a. Samples were then hybridized to duplicate miRNA Matrix® microarrays. Average Cy™3 and Cy™5 signals of the miR-124a features were plotted in comparison to the average Cy™3 and Cy™5 background signals surrounding them. As little as 0.08 fmol of either Cy™3 or Cy™5 labeled synthetic miR-124a RNA was detected over background.

Efficient Labeling and Sensitive Microarray Detection of Endogenous Plant miRNAs and siRNAs

Figure 8. Small RNA samples containing both miRNAs and siRNAs were isolated from two strains of Arabidopsis thaliana, wild type and a dicer mutant strain (dcl1-9). In A. thaliana, dicer (Dcl1) is required for the production of miRNAs but not siRNAs. Thus, the abundance of miRNAs in the dcl1-9 mutant sample is expected to be dramatically reduced in wild type, while endogenous siRNA abundance should not be affected by the dicer mutation. To test this hypothesis, small RNA samples were labeled with Cy™3 using the Label IT® miRNA Labeling Kit and hybridized to duplicate Arabidopsis miRNA-specific microarrays containing custom Arabidopsis siRNA capture sequences (CombiMatrix). Corrected fluorescent signal of each feature was calculated as the ratio of feature fluorescence to mean background fluorescence. Corrected fluorescent signals for each target from duplicate hybridizations were averaged and the ratios of average wild type to average dicer mutant signals were plotted. Ratios of ~1 indicate no difference in miRNA or siRNA abundance between the two samples, however values >1 indicate a decrease in the level of the miRNA or siRNA in the dicer mutant. As expected, miRNA abundance in the dicer mutant was dramatically decreased while the siRNAs should not be affected by the dicer mutation.

Note: The data resulted from a collaboration between Xumei Chen, Ph.D., CombiMatrix, and Mirus Bio. We thank Dr. Chen for sharing the data prior to publication.
Figure 9. miRNA enriched small RNAs from two different samples are labeled with either Cy™3 or Cy™5. Labeled samples are pooled and hybridized to a miRNA-specific microarray. To rule out labeling biases, a dye swap experiment is performed. The same samples are labeled for a second hybridization using the opposite dyes. The microarray slides are scanned and signal measurements from the hybridized samples are analyzed to identify differentially expressed miRNAs. miRNAs that maintain the same expression profile, using each labeling scheme, are further validated.

Figure 10. Gene silencing is triggered by small double-stranded RNAs (dsRNAs), either introduced into the cell by transfection (siRNAs) or arising from nuclear transcripts containing a stem-loop structure. Pre-miRNAs are processed by the RNase III like enzymes, DROSHA in the nucleus and DICER in the cytoplasm, to yield mature miRNAs. siRNAs are derived from large dsRNA molecules in the cytoplasm by the action of DICER. Both miRNAs and siRNAs are incorporated into RISC and depending on the degree of complementarity to the target sequence, are capable of inhibiting translation or initiating cleavage of the target RNA. Generally, miRNAs affect gene expression by inhibiting translation, while siRNAs generally promote target mRNA cleavage and destruction.
miRNA EXPRESSION PROFILING  Convenient Protocol

### RNA REGULATION

#### miRNA Labeling

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#### mirVana™ miRNA Labeling Kit

- Labeling 7 Steps
  - Hyb & Wash
  - Scan

#### NCode™ miRNA Labeling System

- Labeling 5 Steps
  - Hyb & Wash
- Detection 3 Steps
  - Hyb & Wash
  - Scan

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