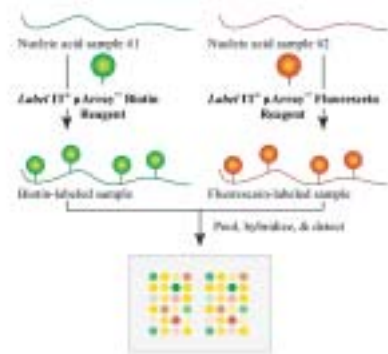
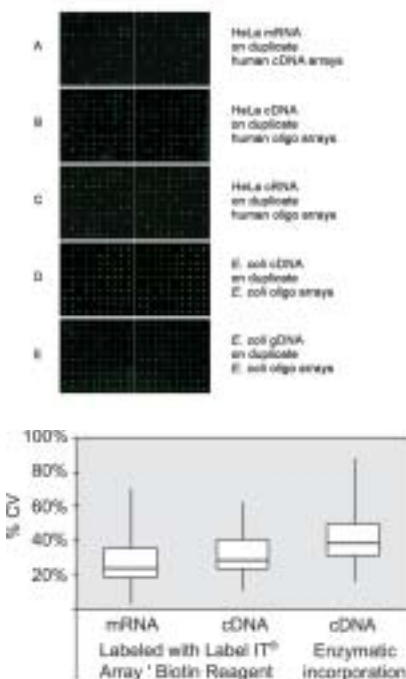


Macro and Micro-Array

Sample Nucleic acid labeling



Overview of the Label IT® μArray™ Dual Labeling Kit



The Label IT labeling kits label any nucleic acid type



To compare the effect of labeling biases on microarray data, brain and liver mRNA samples were each directly labeled for analysis in each channel and pooled appropriately for a «label-swap» experiment using spotted cDNA microarrays (top). Equivalent gene expression ratios were determined for each gene from both analyses (bottom), indicating the reproducibility of gene expression results and the absence of label incorporation bias. In the label-swap experiment, the average log₂ ratio of the Cy5/Cy3 signal ratio for each gene is expected to change sign (e.g. from +1 to -1) when samples are labeled for detection in the opposite channel. Each microarray hybridization contained six replicate features per gene and was conducted in duplicate.

Microarrays represent an established genomics technology that allows thousands of genes to be simultaneously hybridized and quantified in a single experiment. Gene expression profiling, a prominent microarray application, measures the presence and relative amount of specific RNA transcripts from the fluorescent signal generated by the hybridization of a labeled sample to a microarray.

Nucleic acid labeling is required for microarrays, and can be performed via several approaches. This section highlights standard labels (Cy3, Cy5, biotin) as well as selected FluoProbes® products for microarrays applications (preparation of nucleic or protein probes, secondary detection reagent).

Label IT® μArray™ Labeling Kits (Biotin, Dual, Cy™3/Cy™5)

- ◆ Simple, one-step labeling with appropriate dyes (Cy3, CY5, FITC, Biotin)
- ◆ Covalent labeling chemistry eliminates modified nucleotides and enzymatic incorporation
- ◆ Complete protocol for labeling mRNA, cDNA, and cRNA samples is included
- ◆ Detection of rare transcripts and small changes in gene expression
- ◆ Directly label and hybridize nucleic acids of any length

Label IT® μArray™ Labeling Kits offer a convenient, one-step, non-radioactive chemical labeling method of DNA and RNA, optimized for gene expression analysis on microarrays.

Technical tip

Label IT® technology is designed to covalently attach marker molecules (Cy™3, Cy™5, FITC, Biotin) to nucleic acids in a simple one-step non-enzymatic reaction, allowing sensitive and precise detection of labeled samples. It directly labels guanine residues at a site that does not participate in base-pairing during hybridization. As a result, the versatile Label IT® μArray™ Labeling Kits allow any type of nucleic acid sample - mRNA, cDNA, or cRNA - to be directly and uniformly labeled with a marker molecule. They generate high quality microarray hybridization data with low background and high signal-to-noise ratios. Furthermore, the labeling process eliminates biases encountered in alternative methods as labeled nucleotides enzymatic incorporation and as enzymatic labeling.

mRNA samples that are labeled directly with the Label IT® μArray™ Labeling Kits do not require the traditional enzymatic replication step(s): they result in sensitive hybridizations because they represent the original sample without any enzymatic replication or incorporation biases. These directly labeled mRNA allows the detection of low copy number transcripts (less than 10 copies per cell). They also are compatible with hybridization on a variety of microarray surfaces, facilitating substitution into standard protocols

The Label IT® μArray™ Cy™3/Cy™5 Labeling Kit is recommended for one step labeling for dual channel microarray applications. For dual channel applications using Biotin and Fluorescein, use the Label IT® μArray™ Dual Labeling Kit. For other labels and indirect labeling applications, the flexible Label IT® μArray™ Biotin Labeling Kit is available.

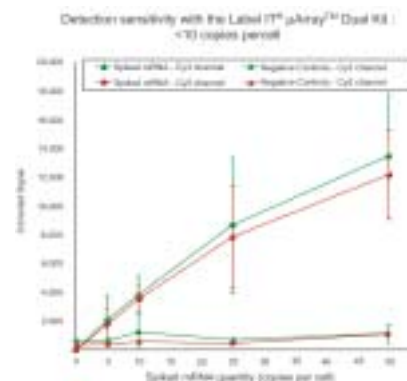
Description	Cat.#	Qty
Label IT® μArray™ Cy™3/Cy™5 Labeling Kit	BJ0600*	2x5 labelings of 1μg nucleic acid
	BJ0600	2x25 labelings of 1μg nucleic acid
Label IT® μArray Dual Labeling Kit (Biotin/FITC)	BA6400*	2x5 labelings of 1μg nucleic acid
	BA6401	2x25 labelings of 1μg nucleic acid
Label IT® μArray Biotin Labeling Kit	AU2540*	10 labelings of 1μg nucleic acid
	AU2541	50 labelings of 1μg nucleic acid

* Each kit includes the Cy3, Cy5, Biotin, or FITC labeling reagent(s), and additional buffers and reagents required for labeling reactions. The protocol provides detailed instructions for labeling various samples for gene expression analysis (mRNA, cDNA, and cRNA). Note: Reagents required for sample preparation, sample purification, microarray hybridization, and secondary detection of biotin are not included.

The **Label IT[®] μ Array[™] Cy[™]3/Cy[™]5 Labeling Kit** allow the direct labeling of nucleic acid samples with the popular Cy[™]3 [$\lambda_{exc.max.}/\lambda_{em.max.}$: 550/570nm] and Cy[™]5 [$\lambda_{exc.max.}/\lambda_{em.max.}$: 649/670nm] fluorophores. These labels can be detected by standard microarray scanners without requiring additional detection reagents or steps. The Label IT[®] μ Array[™] Cy[™]3/Cy[™]5 Labeling Kit provides the optimal labeling density for microarray applications, characterized by high fluorescent signal with minimal quenching.

The **Label IT[®] μ Array[™] Dual Labeling Kit** labels samples with biotin and fluorescein. The use of biotin and fluorescein as marker molecules for hybridization requires a post-hybridization detection procedure with different fluorophore-labeled conjugates. As such, biotin- and fluorescein-labeled samples provide flexibility and compatibility with a variety of established dual color detection strategies. A simple detection method using Cy[™]3-conjugated streptavidin and a Cy[™]5-conjugated anti-fluorescein antibody that is compatible with standard microarray scanners is provided in the kit protocol.

The **Label IT[®] μ Array[™] Biotin Labeling Kit** is recommended for any label with indirect detection microarrays in single and dual color applications. The use of biotin as a marker molecule for hybridization requires a post-hybridization detection procedure with a fluorophore. As such, a biotin-labeled sample provides the flexibility to use any of a variety of established single color detection strategies. In single-color microarray experiments, one sample is hybridized and detected per microarray slide and relative gene expression can be determined from comparison of independently normalized data sets for each sample.



Sensitivity of detection with the **Label IT[®] μ Array[™] Dual Labeling Kit** was determined from spiking experiments in which a specific quantity of an *Arabidopsis* mRNA sample was added to HeLa mRNA before labeling. An estimated mRNA copy number of ~10 copies per cell (30 pg of the spiked mRNA in 1 μ g HeLa mRNA) can be detected in each channel above non-specific hybridization signal (negative controls). Fluorescent signal detected for the spiked mRNA and negative controls is corrected by subtracting the median local background value; error bars represent one standard deviation.

Other labeling reagents

Microarrays are generally performed in single or 2 colors experiments (i.e orange and IR). FluoProbes[®] provides suitable labeling agents, including the standard R-PE, Cy[™]3 and Cy[™]5 labels, and selected FluoProbes[®] alternatives that are recommended for superior results. Streptavidins allows indirect labeling of biotinylated probes and activated dyes suit primary labeling of your specific probes.

Fluorescent Streptavidins for microarray

Description	Cat.#	Qty
Streptavidin-R-Phycoerythrin $\lambda_{abs}/\lambda_{em}$: 564/576 nm Our premium FluoProbes [®] RPE-SAV gives unsurpassed results notably in microarray and ELISA screening.	FP-77776A	1 ml at 1mg/ml
Streptavidin-FluoProbes [®] 488 $\lambda_{abs}/\lambda_{em}$: 593/517 nm A superior alternative to FITC.	FP-20937A	1 mg
Streptavidin-Cy3 [™] $\lambda_{abs}/\lambda_{em}$: 548/562 nm	FP-09825A	1 mg
Streptavidin-FluoProbes [®] 547 $\lambda_{abs}/\lambda_{em}$: 545/561 nm A superior alternative to Cy [™] 3.	FP-AX1460	1 mg
Streptavidin-Cy5 [™] $\lambda_{abs}/\lambda_{em}$: 646/664 nm	FP-28407A	1 mg
Streptavidin-FluoProbes [®] 630 $\lambda_{abs}/\lambda_{em}$: 630/657 nm A superior alternative to Cy [™] 5.	FP-AU2870	1 mg
Streptavidin-FluoProbes [®] 647 $\lambda_{abs}/\lambda_{em}$: 652/673 nm A new alternative to Cy [™] 5.	FP-BA1270	1 mg

Note : additional information can be found in sections II.A, B for the labels, and III.B for streptavidins.

Fluorescent activated dyes

Description	Cat.#	Qty
FluoProbes [®] 647-NHS $\lambda_{abs}/\lambda_{em}$: 652/673 nm ; EC : 250 000 M ⁻¹ cm ⁻¹ FluoProbes [®] 647 NHS provides a high labeling efficiency. Compared to the Alexa Fluor [®] 647, FluoProbes [®] 647 has been used successfully to increase the quantity of dye linked to the target (more than 10% of dye after labeling). Also FluoProbes [®] 647 shows extended signal emission time. In regards of its competitive price, it will be the right choice for routine application with a single dye like Cy [™] 5.	FP-AK7740	1 mg
FluoProbes [®] 630-NHS $\lambda_{abs}/\lambda_{em}$: 636/657 nm ; EC : 200 000 M ⁻¹ cm ⁻¹ An excellent label, alternative to Cy [™] 5 (see technical tip below).	FP-AM7580	1 mg
FluoProbes [®] 547-NHS $\lambda_{abs}/\lambda_{em}$: 557/574 nm Superior alternative to Cy [™] 3.	FP-AK7730	1 mg

See also :

Page D.135 : labeled nucleotides (for labeling by situ PCR incorporation)

Chapter B (Proteomics) : fluorescent and biotin labeling agents

Page A.350 : (strep)avidin reagents (for biotin detection)

Associated products:

Streptavidin-FP488 FP-BA2221

and other streptavidin reagents (see page A.350)

See also our FluoProbes[®] 546 and 642 labeled immunologicals for protein microarrays.

Other FluoProbes[®] dyes are available !

Our FluoProbes[®] labeling agents are described in section Biochemistry.

Our FluoProbes[®] labeled immunologicals (II Abs, Streptavidin) are presented in Chapter A.

Macro and Micro-Array

Sample Nucleic acid labeling

Researchers Discover the Advantages of Making the Switch

Quality control of chip manufacture and chip analysis using epoxy-chips as a model

[...] We tested an alternative fluorescent label : FluoProbes® 630-NHS, designed for excitation by He/Ne- and diode-lasers. To compare the extent of incorporation of Cy5 and FluoProbes® 630, 0.3, 0.5 and 1 ml extracted *Rhizobium fredii* DNA were used as template for the PCR-reaction. As can be seen from figure below, FluoProbes® 630 led to a higher yield of labeled DNA. Thus, from the same starting amount of DNA, amplified DNA with a higher level of label incorporation and therefore higher fluorescence can be produced. As a result, less DNA is needed for chip analysis that is of great importance especially in medical diagnostics and cancer research, where probe material is very limited.



PCR in situ labeling : DNA/RNA are efficiently labeled during the amplification step using aminoallyl, or fluorescent directly labeled nucleotides.

Fluorescent activated dyes

Description	Cat.#	Qty
FluoProbes® 647-NHS $\lambda_{abs}/\lambda_{em.}$: 652/673 nm ; EC : 250 000 M ⁻¹ cm ⁻¹ FluoProbes® 647 NHS provides an high labeling efficiency. Compared to the competitor 647 dye, FluoProbes® 647 has been used successfully to increase the quantity of dye linked to the target (more than 10% of dye after labeling). Also FluoProbes® 647 shows extended signal emission time. In regards of its competitive price, it will be the right choice for routine application with a single dye like Cy™5.	FP-AK7740	1 mg
FluoProbes® 630-NHS $\lambda_{abs}/\lambda_{em.}$: 636/657 nm ; EC : 200 000 M ⁻¹ cm ⁻¹ An excellent label, alternative to Cy™5 (see technical tip below).	FP-AM7580	1 mg
FluoProbes® 547-NHS $\lambda_{abs}/\lambda_{em.}$: 557/574 nm Superior alternative to Cy™3.	FP-AK7730	1 mg

MicroArray Hybridization

FluoProbes provides accessory tools, solutions below and chambers/gaskets (page D.127) for improved hybridization.

Microarray Hybridization Buffers

These general hybridization buffers require no preparation. They are ready to use, pre-mixed (and at various concentrations) for many different research needs, including microarray and hybridization. They have optimized formulations. These General Hybridization Buffers are ideal for such specific applications as hybridization, blotting, binding, and washing. The General Hybridization Buffers are manufactured using only the highest quality reagents and are filtered to provide high sensitivity and a very low background every time they are used. The buffers are available as here after :

Description	Cat.#	Qty
Microarray Hybridization Buffer 0.1 x SSC Buffer W/0.2 % SDS	FP-BA9630	1 L
Microarray Hybridization Buffer 0.1 x SSPE Buffer W/0.2 % SDS	FP-BA9640	1 L
Microarray Hybridization Buffer 10 x SSC Buffer W/0.5 % SDS	FP-BA9660	1 L
Microarray Hybridization Buffer 1 x SSC Buffer W/0.1 % SDS	FP-BA9610	1 L
Microarray Hybridization Buffer 20 x SSC Buffer W/1 % Sarcosyl	FP-BA9670	1 L
Microarray Hybridization Buffer 6 x SSC Buffer W/0.2 % SDS	FP-BA9620	1 L