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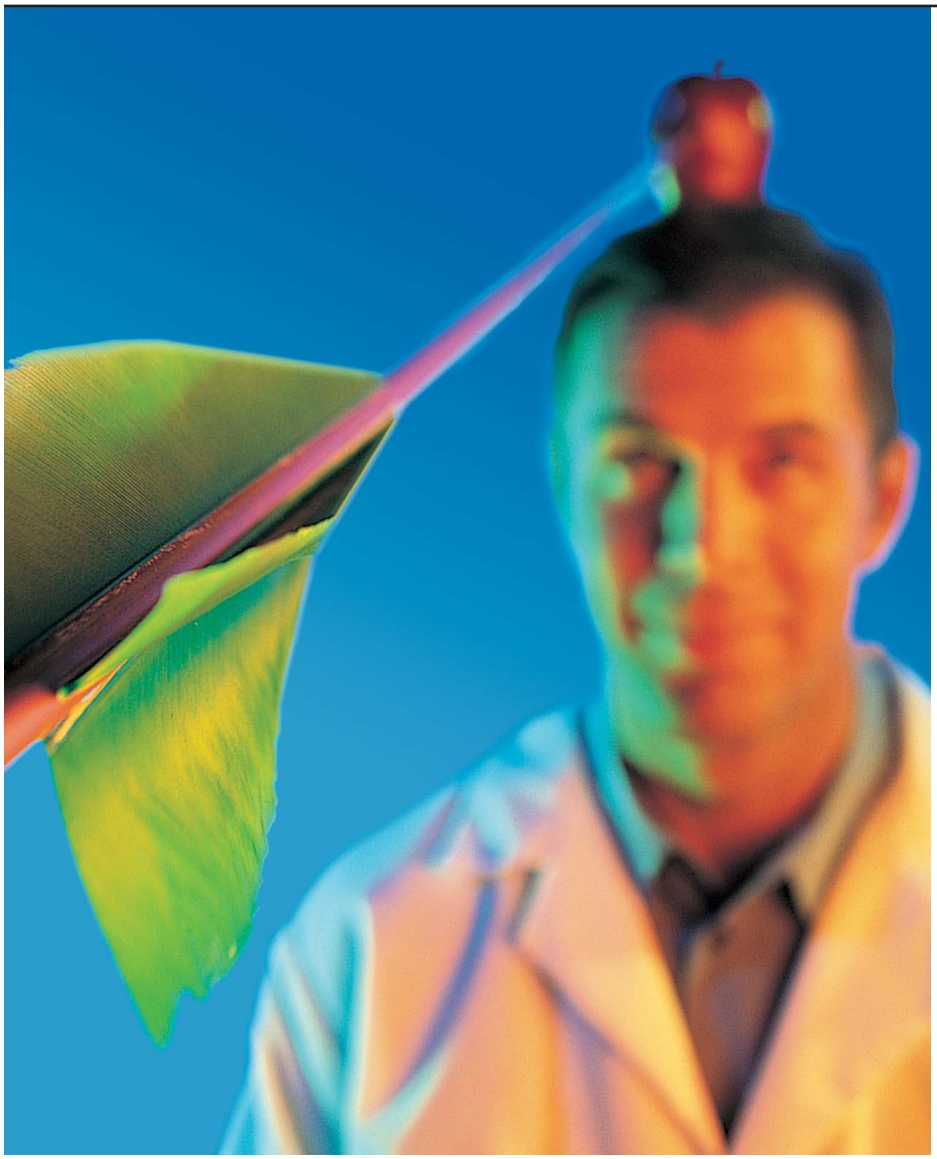
Microarray target labeling
you can trust



- **Performance**–high cDNA yields and bright signals increase sensitivity
- **Reproducibility**–optimized system ensures consistent performance
- **Convenience**–includes all the major labeling components in one kit, simplifying ordering

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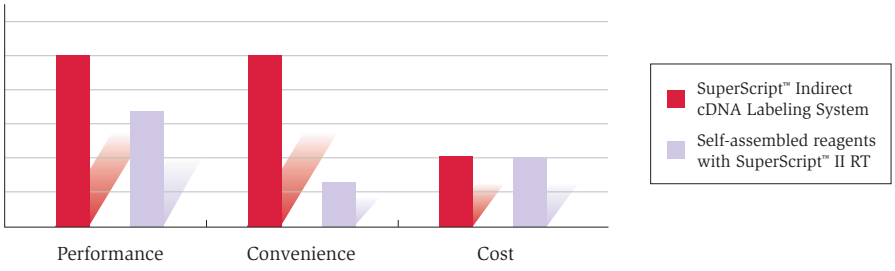
Highly sensitive detection for accurate microarray analysis

For accurate and dependable microarray target labeling you don't need to practice. The SuperScript™ Indirect cDNA Labeling System is based on proven methods of indirect cDNA labeling for microarray analysis. Get high cDNA yields using SuperScript™ III Reverse Transcriptase (RT) and increased signal intensity from a proprietary amino-modified nucleotide mixture in one convenient kit. You'll improve your sensitivity and never go back to your old labeling methods again.

No assembly required

The SuperScript™ Indirect cDNA Labeling System is an array labeling kit based on proven methods of indirect cDNA labeling. The optimized system provides SuperScript™ III RT for generating high cDNA yields, a proprietary nucleotide mixture to increase signal intensity, and a convenient kit format that saves you valuable time. You'll no longer have to source and optimize your reagents, and you won't have to adjust to any new methods. It's extra convenience that comes at the same price—without all the extra work (Figure 1). You can maintain a cost-effective approach to your microarray studies and get the unparalleled results that only SuperScript™ III RT can deliver.

Figure 1 - Convenience and improved performance with SuperScript™ Indirect cDNA Labeling System



Novel nucleotide mix increases signal intensity

Most indirect labeling kits use an amino-allyl-modified nucleotide during first-strand cDNA synthesis. This modified nucleotide can be effectively "post-labeled" or coupled with an NHS-ester form of Cy™3/Cy™5 or a range of other commercially available dyes. The SuperScript™ Indirect System takes it one step further, using two modified nucleotides during this stage. You'll get more efficient and even incoporation of amino-modified nucleotides throughout your cDNA targets. Using the SuperScript™ Indirect cDNA Labeling System, your labeled cDNA targets will display improved hybridization efficiency and signal intensity. This unique amino-allyl/aminohexyl (AA/AH) nucleotide mixture increases signal intensity four-fold over the self-assembled methods and well exceeds that of other commercial kits (Figures 2 and 3), enabling greater sensitivity. You'll be able to monitor expression more effectively, improving your results.

Figure 2 - Greater signal intensity and signal-to-noise ratio with the SuperScript™ Indirect System compared to self-assembled method

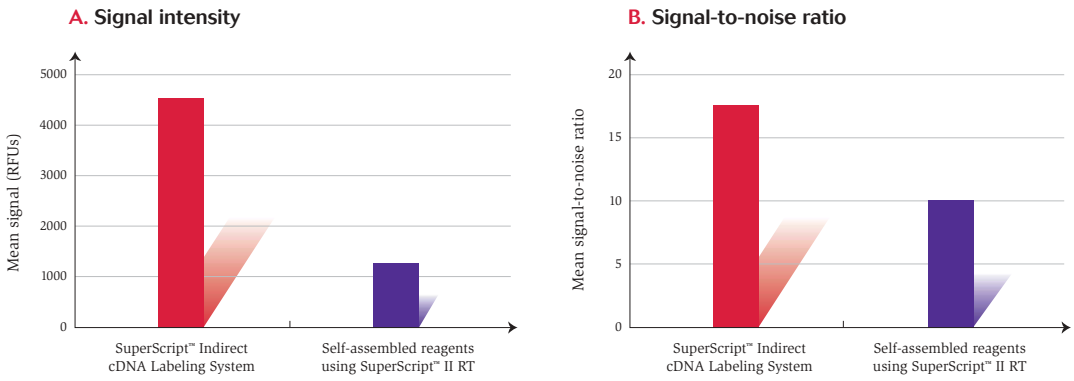
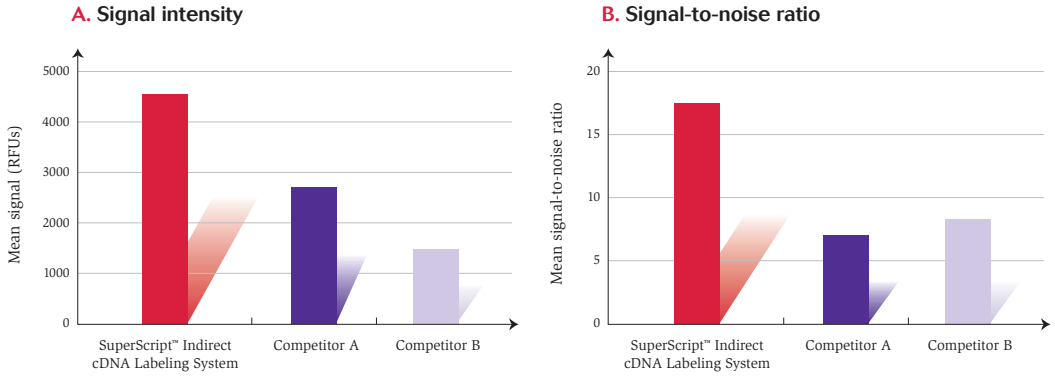


Figure 3 - The SuperScript™ Indirect cDNA Labeling System gives you brighter signals and less noise than the competition

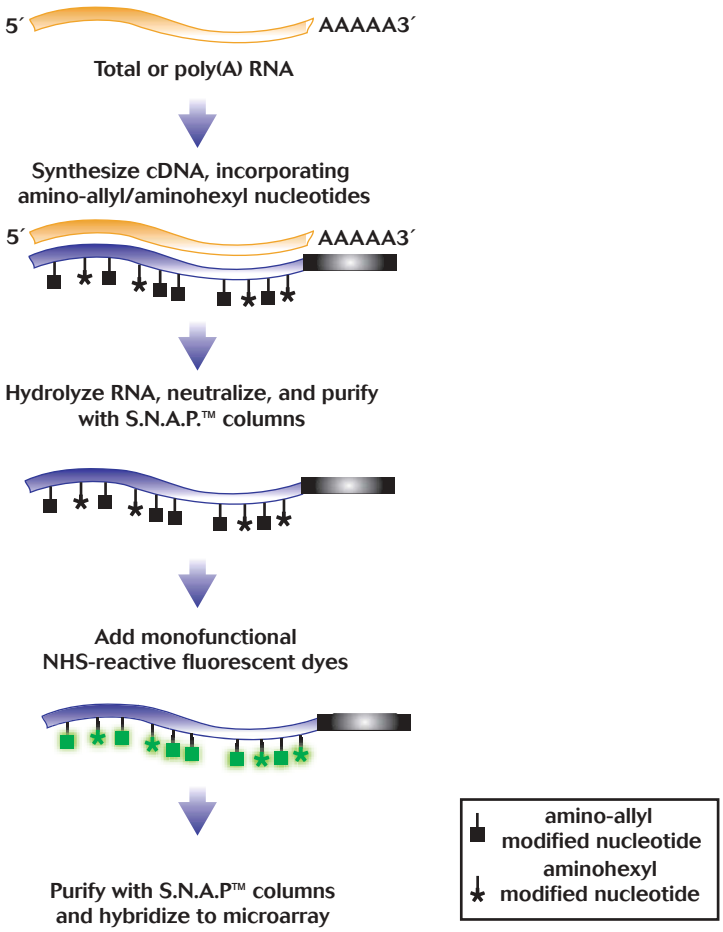


Analysis of arrays (MWG Human Starter) hybridized with Cy™3-labeled cDNA prepared using the SuperScript™ Indirect cDNA Labeling System, common protocol using self-assembled reagents with SuperScript™ II RT and competitor's kits. Triplicate labeling reactions were performed and hybridized to array. Graphs 2A/3A shows the mean signal intensity of all positive genes for the average of the triplicate reactions. Graphs 2B/3B shows the mean signal-to-noise ratios.

Proven protocol

The SuperScript™ Indirect cDNA Labeling System protocol is based on proven methods for indirect cDNA labeling for microarray analysis. As shown in Figure 4, mRNA or total RNA is first reverse transcribed using SuperScript™ III RT, incorporating two amino-modified nucleotides into the synthesized cDNA. The template RNA is then degraded by base hydrolysis, and the reaction is neutralized with acid. The amino-modified cDNA is then purified to remove unincorporated nucleotides, primers, and buffers. In the second step, the modified cDNA is coupled with the active form of a fluorescent dye. The fluorescently labeled cDNA is purified with a S.N.A.P.™ spin column to remove any unreacted dye. The resulting fluorescently labeled cDNA is ready for hybridization to microarrays.

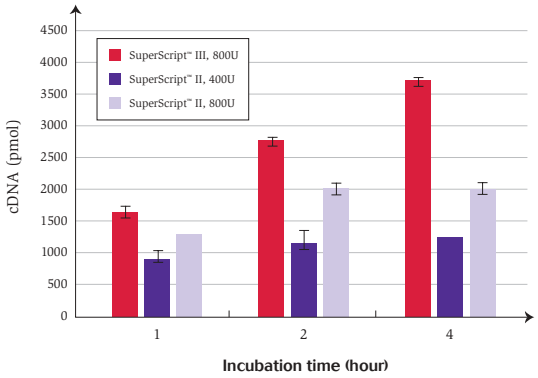
Figure 4 - SuperScript™ Indirect System follows proven protocols



Higher yields with SuperScript™ III RT

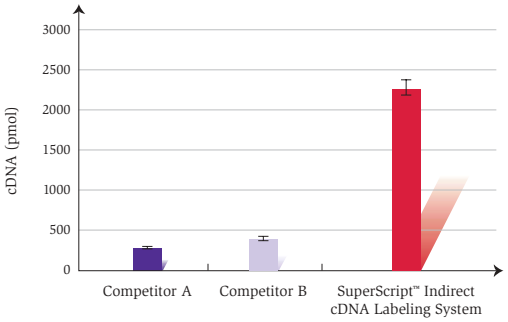
The SuperScript™ Indirect cDNA Labeling System was developed with SuperScript™ III RT to improve microarray labeling results. SuperScript™ III RT is a point mutant of SuperScript™ II resulting in a longer half life, allowing for extended incubations and higher cDNA yields. Combined with a high concentration of 400 units/µl, the longer half-life allows you to achieve twice the cDNA of current methods employing SuperScript™ II RT (Figure 5), and far more than the competition (Figure 6). There's no longer a need for an additional "boost" of enzyme during your labeling procedure to obtain high yields. With higher cDNA yields, you'll get improved gene representation and increased sensitivity in your experiments.

Figure 5 - SuperScript™ III generates higher cDNA yields than SuperScript™ II RT



cDNA was synthesized with different amounts of SuperScript™ III and SuperScript™ II RT. The reaction mixtures included 1X first-strand buffer, 5 mM DTT, 10 µg of total RNA, 2 µg oligo(dT)$_{20}$, 40 U RNaseOUT™, 0.5 mM dNTP with amino-allyl and aminohexyl replacement, and 1 µCi [α-^{32}P]dCTP. 5 µl of the reaction was removed and TCA-precipitated. CPM were counted.

Figure 6 - SuperScript™ Indirect cDNA Labeling System generates higher cDNA yields than the competition

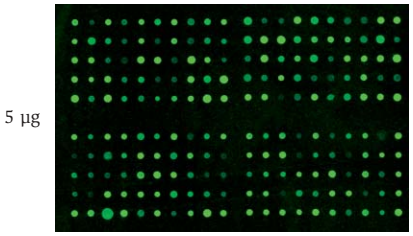


cDNA was synthesized using the SuperScript™ Indirect cDNA Labeling System and commercial kits according to manufacturer's protocols from 10 µg Hela total RNA. In each reaction, ^{32}P-α-dCTP was added to trace the cDNA synthesis and 20% of the reaction mixture was spotted on GF/C filter and cDNA yield was calculated according to TCA-precipitated ^{32}P counts.

Sensitivity from 5 µg total RNA

With increased cDNA yields over SuperScript™ II, SuperScript™ III RT allows you to use less starting material for increased sensitivity. You can successfully perform microarray hybridizations using as little as 5 µg of total RNA (Figure 7). You'll obtain labeled targets that are more representative of the expressed transcripts in your sample, allowing you to monitor differentially expressed genes with greater sensitivity.

Figure 7 - Microarray hybridizations using the SuperScript™ Indirect cDNA Labeling System and 5 µg of pooled total RNA



Array (MWG Human Starter) was hybridized overnight with Cy™3-labeled cDNA prepared from 5 micrograms of pooled human total RNA using the SuperScript™ Indirect cDNA Labeling System. The image was acquired using Axon's GenePix® 4000B microarray scanner.

It's everything you need for reproducible labeling

The SuperScript™ Indirect cDNA Labeling System comes with all the major reagents* to effectively produce labeled cDNA targets for microarray hybridization (Table 1)—all functionally tested for quality to ensure reproducible fluorescent labeling. Armed with high-quality reagents and an optimized protocol, you'll obtain highly fluoresent cDNA targets, consistently.

Table 1 - SuperScript™ Indirect cDNA Labeling System components*

SuperScript™ III RT (400 U/µl)	10 mM dNTP Mix	Glycogen (20 mg/ml)
Anchored Oligo $(dT)_{20}$ (2.5 µg/µl)	RNaseOUT™ (40 U/µl)	S.N.A.P™ Columns
Random Primers	DEPC-water	Collection Tubes
Control RNA Ladder (0.5 µg/µl)	DMSO (Labeling Grade)	Loading Buffer
5X First-strand Buffer	2X Coupling Buffer	Wash Buffer
0.1 M DTT	3 M NaAc, pH 5.2	Amber Collection Tubes

* Monofunctional NHS-reactive fluorescent dyes not included.

Get better results, easier

Target labeling is an integral step in obtaining accurate results during microarray experiments. Don't compromise on sensitivity. The SuperScript™ Indirect cDNA Labeling System provides a convenient means to increase the sensitivity in your experiments, giving you better results. Call and order today.

Description	Quantity	Cat. no.
SuperScript™ Indirect cDNA Labeling System	10 reactions	L1014-01
	30 reactions	L1014-02
Anchored Oligo $(dT)_{20}$ Primer	50 µg	18418-038

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