QIAGEN News
Innovation Working for You

4
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New

LabelStar™ Array Kit — efficient cDNA labeling and cleanup for high signal-to-noise ratios on arrays

- **High signal intensity and low background** — identification of true positives at low expression levels
- **Labeling using a wide range of RNA amounts** — from just 0.2 µg to 50 µg RNA
- **Flexibility in choice of label** — incorporation of any commonly used modified nucleotide
- **Optimized labeling and cleanup procedures** — reproducible, high-quality results
- **A fast and easy system** — reliable performance

▶▶ New LabelStar Array Kit, page 8

Coming soon!

PAXgene™ Blood DNA System — a standardized system for blood collection and genomic DNA isolation

- **Integrated, standardized system** — blood collection, transport, and storage, with DNA purification in one system
- **Convenient storage** — blood can be stored in PAXgene™ Blood DNA Tubes for up to 10 days at room temperature before DNA purification
- **Enhanced workflow efficiency**
- **Easy handling** — purification in a single tube minimizes the risks of sample mix-up and cross-contamination, and reduces plasticware consumption
- **Rapid** — only 1 hour of hands-on time for DNA purification from 12 samples
- **High yields of pure, high-molecular-weight DNA** — up to 500 µg of DNA from each sample

▶▶ PAXgene Blood DNA System, page 17

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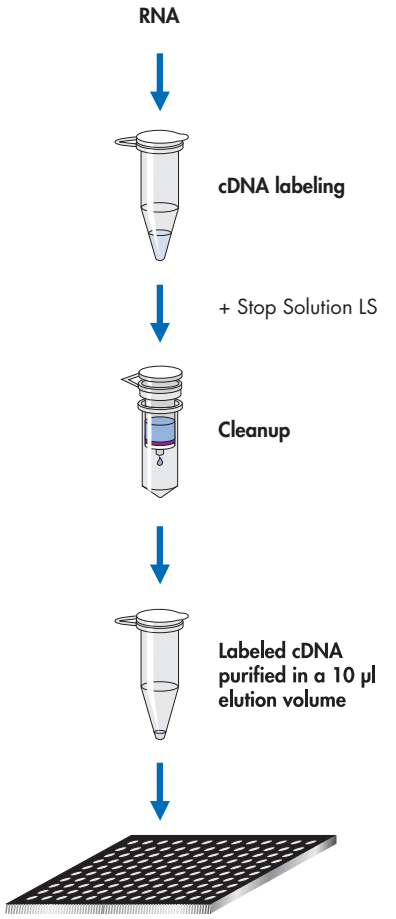
LabelStar Array Procedure



continued from page 1

Efficient cDNA labeling and cleanup

The new LabelStar™ Array Kit provides efficient labeling and cleanup of cDNA before array hybridization. Optimized reaction conditions ensure high signal intensity and low background. Reproducible and robust labeling over a range of RNA amounts and a wide choice of labels results in a flexible easy-to-use system. The LabelStar Array Kit allows highly sensitive array analysis through identification of true positives at low gene expression levels.

The rapidly expanding field of microarray analysis has led to many important advances in gene expression profiling. To meet the demands of this rapidly expanding field, more robust and sensitive technology is required. The most important factor for sensitivity is the efficiency of the labeling reaction, which can be compromised by inhibitors of reverse transcription that are copurified with the RNA and by inefficient incorporation of the modified nucleotide. Following the labeling reaction, removal of unincorporated nucleotides is necessary to reduce background and increase signal-to-noise ratios on the chip. Both labeling and cleanup are therefore required to maximize sensitivity of array analysis.

The LabelStar procedure

The LabelStar Array Kit combines efficient cDNA labeling, followed by cleanup, with high DNA recovery in a few simple steps (see flowchart). The LabelStar Array Kit is comprised of two modules. The cDNA Labeling Module contains LabelStar Reverse Transcriptase, dNTPs, RNase inhibitor, and all buffers and solutions required for labeling (excluding labeled nucleotides). The Cleanup Module contains MinElute™ spin columns and buffers optimized for cleanup of labeled cDNA used in array analysis.

During the LabelStar procedure, isolated RNA is denatured using Denaturation Solution Plus. This solution also neutralizes inhibitors of reverse transcription copurified with the RNA. During reverse transcription of the denatured RNA using LabelStar Reverse Transcriptase, a modified nucleotide of choice is incorporated (Table 1). For high signal intensity, degradation of remaining RNA is necessary after the labeling reaction. The exoribonuclease activity of LabelStar Reverse Transcriptase efficiently degrades RNA, eliminating the need for a separate degradation step. Stop Solution LS stops the labeling reaction and reduces nonspecific binding of components of the labeling reaction to the array, reducing background signal.

Table 1. Specifications

Amount of total RNA used in labeling reaction	**0.2–50 µg**
Amount of mRNA used in labeling reaction	**0.2–5 µg**
Modified nucleotides suitable for cDNA labeling using the LabelStar Array Kit:	
Cyanine-3/Cyanine-5-dCTP	✔
Cyanine-3/Cyanine-5-dUTP	✔
Biotin-dCTP or -dUTP	✔
5-(3-aminoallyl)-2'-dUTP	✔
^{32}P-dCTP	✔
^{33}P-dCTP	✔
Final elution volume	**10 µl**

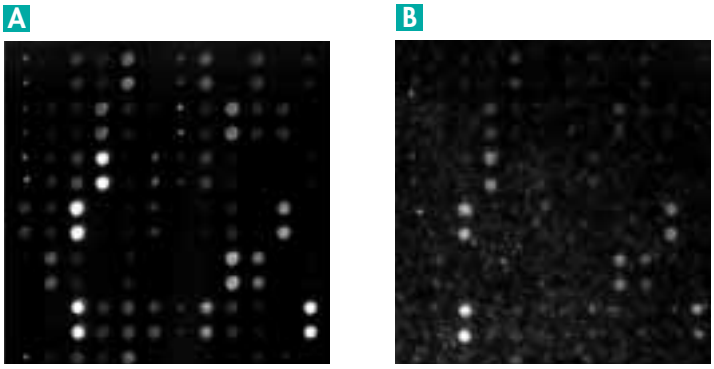
Optimized cleanup procedure

Cleanup of labeled cDNA is performed with silica-membrane–based MinElute spin columns using a simple bind–wash–elute procedure. All contaminants from the labeling procedure, such as unincorporated nucleotides, proteins, or salts are efficiently removed in the cleanup procedure. Optimized buffer sets and a novel spin-column design ensure high recovery and high purity of labeled cDNA in low elution volumes. Compared with other methods (e.g., ultrafiltration), use of the LabelStar Cleanup Module results in reproducibly higher signal-to-noise ratios (Figure 1). In addition, the very low elution volume of 10 µl often eliminates the need for further concentration of labeled cDNA before array hybridization. cDNA labeled and purified using the LabelStar Array Kit is ready for hybridization to arrays, independent of the type of probe (e.g., oligos, PCR fragments) or support material (e.g., glass slides, membrane arrays).

High signal intensities

The LabelStar Array Kit offers a labeling and cleanup system that can be adapted for direct and indirect cDNA labeling using a wide range of modified nucleotides and amounts of RNA (Table 1). Using the LabelStar Array Kit, higher signal intensities and lower background are obtained (Figure 2). The LabelStar procedure results in up to ninefold higher signal intensities, compared with other commercially available kits (Figure 3). The LabelStar Kit enables the detection of more true positive spots when using low amounts of RNA and at low levels of gene expression (Figure 1). ▶

Optimized LabelStar Cleanup Procedure



LabelStar Cleanup Module Supplier M_{III}

Figure 1 *cDNA labeling was performed using 20 µg of total RNA from mouse brain using the LabelStar cDNA Labeling Module. Purification of the labeled cDNA was performed using* **A** *the LabelStar Cleanup Module or* **B** *a commonly used ultracentrifugation-based method from Supplier M_{III}. Purified cDNAs (1/10 total volume) were hybridized to QIAGEN SensiChip™ DNA Arrays carrying a set of mouse genome oligonucleotide probes. Hybridization and washing procedures were identical in both cases.*

More Positives and Higher Signal-to-Noise Ratio with the LabelStar Array Kit

LabelStar Supplier L Supplier I Supplier A_{II} Supplier S_{III}

Figure 2 *cDNA labeling (with Cy®3) and purification was performed using 20 µg total RNA from HeLa cells using the LabelStar Array Kit* ***(LabelStar)*** *or kits from the 4 suppliers indicated, following the manufacturers' instructions. Each labeled and purified cDNA preparation was hybridized to a chip containing a set of human genome oligonucleotide probes. Hybridization and washing conditions were the same on all chips.*

Higher Signal Intensities Using the LabelStar Array Kit

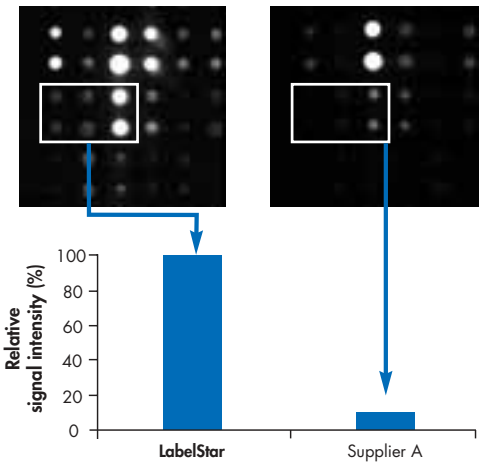


Figure 3 *cDNA labeling (with Cy5) and purification were performed using 2 µg total RNA from mouse brain using the LabelStar Array Kit and a kit from Supplier A, following the manufacturers' instructions. The signal intensities of six spots were compared. Mean values of relative signal intensities were calculated (see bar graph). On average, signal intensities were ninefold higher using the LabelStar Array Kit.*

Summary

The LabelStar Array Kit provides a complete solution for highly efficient labeling and purification of cDNA generated by reverse transcription using 0.2–50 µg of RNA. Compared with other methods, cDNA labeled using the LabelStar Array Kit gives higher signal intensities and lower backgrounds on chips, yielding more true positive spots. This is a result of optimized conditions in the labeling reaction and subsequent purification. ■

Ordering Information

Product	Contents	Cat. No.
LabelStar Array Kit (12)	For 12 labeling reactions: LabelStar Reverse Transcriptase, dNTPs,* RNase Inhibitor, Oligo-dT Primer, 12 MinElute Spin Columns, RNase-Free Reagents, Buffers	28902
LabelStar Array Kit (50)	For 50 labeling reactions: LabelStar Reverse Transcriptase, dNTPs,* RNase Inhibitor, Oligo-dT Primer, 50 MinElute Spin Columns, RNase-Free Reagents, Buffers	28904

** 20 mM solutions of each dNTP; labeled nucleotides to be supplied by user*

"Clone blunt-ended PCR products easily and efficiently with the QIAGEN A-Addition Kit", page 7

Product	Contents	Cat. No.
QIAGEN A-Addition Kit	For 40 A-addition reactions: 5x QIAGEN A-Addition Master Mix, Nuclease-free water	231994
Related products		
QIAGEN PCR Cloning Kit (10)†	For 10 reactions: 2x Ligation Master Mix (50 µl), pDrive Cloning Vector (0.5 µg), distilled water (1.7 ml)	231122
QIAGEN PCR Cloning^{plus} Kit (10)†	For 10 reactions: 2x Ligation Master Mix (50 µl), pDrive Cloning Vector (0.5 µg), distilled water (1.7 ml), QIAGEN EZ Competent Cells (10 tubes, 50 µl each), SOC medium (2 x 1.9 ml)	231222
ProofStart DNA Polymerase (500 U)	500 units ProofStart DNA Polymerase, 10x ProofStart PCR Buffer,‡ 5x Q-Solution, 25 mM $MgSO_4$	202205

† Larger kit sizes available; please inquire.
‡ Contains 15 mM $MgSO_4$.