# Applied Biosystems cRNA and cDNA Labeling and Chemiluminescent Detection Kits

- **Improved *in vitro* transcription amplification protocol for labeling very low amounts of RNA**
- **Three options for digoxigenin labeling provide greater flexibility**
- **Chemistries optimized to provide chemiluminescent signal for highly robust detection of gene expression levels**


## Introduction

Applied Biosystems provides three kits for use with the Applied Biosystems Expression Array System. All three kits give you the highest quality gene expression results available today at the sensitivities you require. Each labeling kit accurately converts total RNA into either labeled cRNA or cDNA for sensitive chemiluminescent detection of gene expression levels that result in maximal signal intensities. The kits include:

- **NanoAmp™ RT-IVT Labeling Kit** Converts the mRNA fraction of total RNA into labeled cRNA using T7-based linear amplification. The NanoAmp Kit has double-IVT capability (i.e., two rounds of *in vitro* transcription amplification) for exceptionally low starting amounts.
- **Chemiluminescent RT Labeling Kit** Converts the mRNA fraction of total RNA to labeled cDNA.
- **Chemiluminescence Detection Kit** Uses robust chemiluminescence chemistry to report the hybridization of digoxigenin (DIG)-labeled cRNA or cDNA to probes attached to the microarray

## Overview of the NanoAmp™ RT-IVT and Chemiluminescent RT Labeling Kits

The RT-IVT and RT labeling kits are optimized for maximal incorporation of label into the target sequence. The choice of kit is determined by the quantity of available sample as seen in Table 1. Both kits use mutant forms of Moloney Murine leukemia virus reverse transcriptase (MMLV RT) that have been specifically optimized to produce high yields and high fidelity cDNA synthesis.

### 1.NanoAmp™ RT-IVT Labeling Kit

The Applied Biosystems NanoAmp™ RT-IVT Labeling Kit enables researchers with only small quantities of total RNA to prepare adequate quantities of DIG-labeled cRNA

**Table 1. Comparison of Total RNA Input for Labeling Reactions**

| Kit | Starting amount of total RNA | Protocol |
|---|---|---|
| NanoAmp™ RT-IVT Labeling Kit | As low as 200 ng | *Single IVT Labeling |
| NanoAmp™ RT-IVT Labeling Kit | As low as 5 ng | **Double IVT Labeling |
| Chemiluminescent RT Labeling Kit | 40 µg | |

*One round of *in vitro* transcription amplification. **Two rounds of *in vitro* transcription amplification.

suitable for subsequent gene expression analysis (Figure 1). The chemistry of the NanoAmp Kit, which utilizes the Eberwine linear amplification procedure[1], increases the yield of cRNA from cDNA > 1,000-fold in a single-round of amplification.

For extremely limited RNA quantities, a single-round of amplification may not generate sufficient cRNA for hybridization. In these situations a second round of amplification can be performed using the first-round cRNA as a template. This produces an approximate $10^6$-fold increase from total RNA.

## The RT-IVT Labeling Process

### Single IVT Labeling

RT-IVT labeling begins with the synthesis of cDNA from the RNA sequence by means of an oligo (dT) primer linked to an RNA polymerase promoter region. Antisense RNA is then transcribed from the cDNA by T7 RNA polymerase, which also incorporates a DIG-modified nucleotide (Figure 2).

### Double IVT Labeling

For studies in which the sample amount is severely limited, a second round of amplification may be required. A portion of the cRNA from the first round of amplification can be used as template for the second round of cDNA synthesis and T7 RNA polymerase amplification. Specialized oligos are used to prime the first strand cDNA synthesis from the cRNA templates. The second *in vitro* transcription reaction incorporates the DIG label for subsequent chemiluminescence detection.

### Typical RT-IVT Yields

Single IVT reactions typically yield approximately 30 µg of labeled cRNA from a starting sample of 200 ng of total RNA. Double IVT reactions as low as 5 ng of total input RNA typically yields sufficient cRNA for microarray experiments. A minimum of 10 µg of labeled cRNA per microarray hybridization is required for optimal gene expression analysis. However, the major factors affecting overall yield are the tissue source and the quality of the extracted total RNA.

## 2. Chemiluminescent RT Labeling Kit

The Applied Biosystems Chemiluminescent RT Labeling Kit converts mRNA into labeled cDNA. Although the labeling method does not employ amplification, the sensitivity of the Applied Biosystems Expression Array System enables the detection of approximately one to two copies of mRNA per cell. When the starting amount of RNA is not limited, this non-amplification method (RT labeling)

## Single RT-IVT Yields

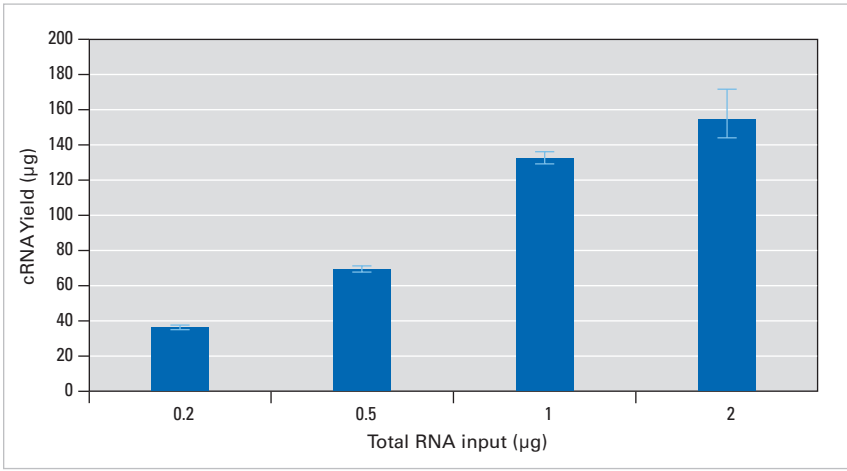


**Figure 1.** Single IVT yields using NanoAmp™ RT-IVT Labeling Kit with Universal Human Reference RNA (Stratagene)

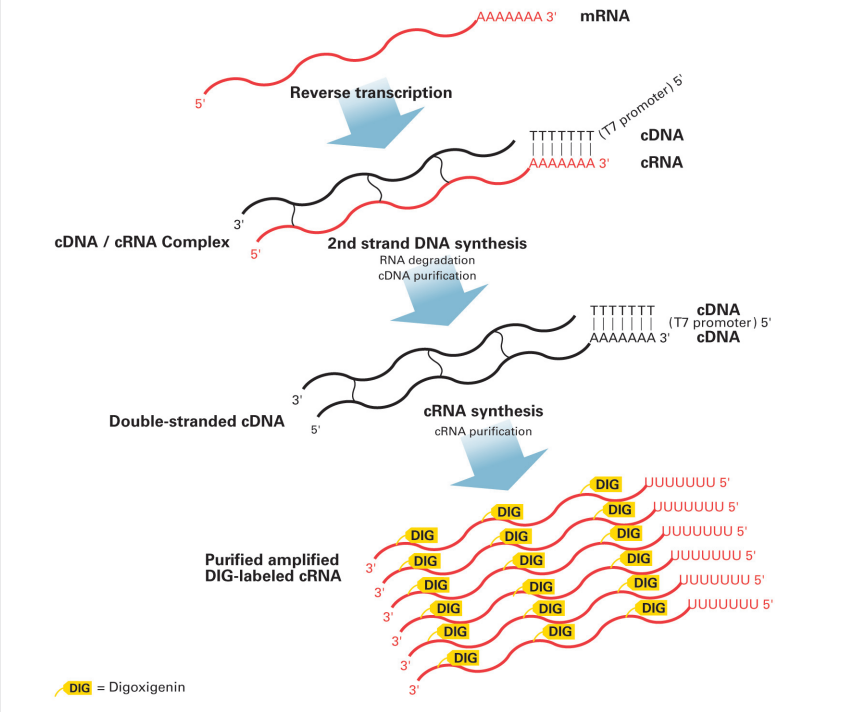


**Figure 2.** Schematic of the RT-IVT labeling process used in the Applied Biosystems NanoAmp Labeling Kit

is very rapid, compared to the amplification (RT-IVT) protocol.

### The RT Labeling Process

The RT labeling process begins with reverse transcription of poly(A) mRNA, using an oligo (dT) primer (Figure 3). The polymerization reaction incorporates nucleotides modified with digoxigenin. After the mRNA is hydrolyzed, the purified DIG-labeled cDNA is ready for hybridization on Applied Biosystems Genome Survey Microarrays.

### Labeling Kit Components

Each kit includes almost everything needed for high-yield RT-IVT and RT reactions. The exceptions are DIG-UTP, which is required for the RT-IVT kit, and DIG-dUTP, required for RT labeling. Both reagents are available from Roche Applied Science (see ordering information for details). The NanoAmp™ Kit contains sufficient reagents for 12 single IVT reactions or six double IVT reactions. The Chemiluminescence RT Labeling Kit is configured for 12 cDNA synthesis reactions. Both kits contain all the controls required to trouble shoot unsuccessful labeling reactions.

## Choosing the Correct Labeling Kit

The Applied Biosystems NanoAmp™ RT-IVT Labeling Kit and Chemiluminescent RT Labeling Kit both produce high-quality DIG-labeled cRNA or cDNA, respectively, and are optimized for the Applied Biosystems Expression Array System. Table 1 provides the recommended labeling kit and protocol to generate sufficient labeled material for microarray hybridization, based on the amount of total RNA available.

## 3. Chemiluminescence Detection Kit

The Applied Biosystems Chemiluminescence Detection Kit provides reagents for the hybridization and chemiluminescent detection of DIG-labeled targets on the microarray (Figure 4). A novel derivative of the CDP-*Star*® chemiluminescent substrate[2], optimized for signal strength, was developed expressly for this application. The microarrays are subsequently analyzed on the Applied Biosystems 1700 Chemiluminescent Microarray Analyzer and provide the highest level of sensitivity.

### Light Production

The production of light reaches a steady state in approximately five minutes and emits light for > 60 minutes without significant changes in intensity (Figure 5). Excellent signal-to-noise ratios are produced for very low gene expression levels due to the highly reproducible chemiluminescent chemistry and the absence of an excitation background. This performance is far superior to that of alternative microarray systems that use limited, typically fluorescence-based detection methods.

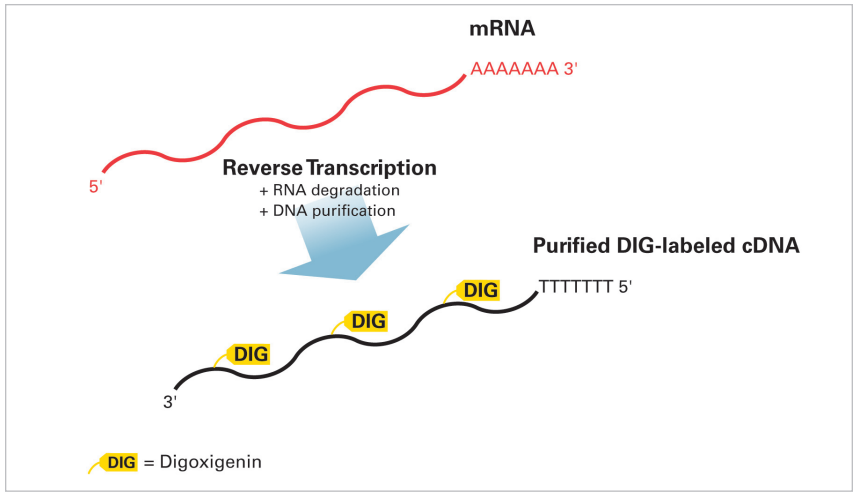


**Figure 3.** Schematic of the RT labeling process used in the Applied Biosystems Chemiluminescent RT Labeling Kit

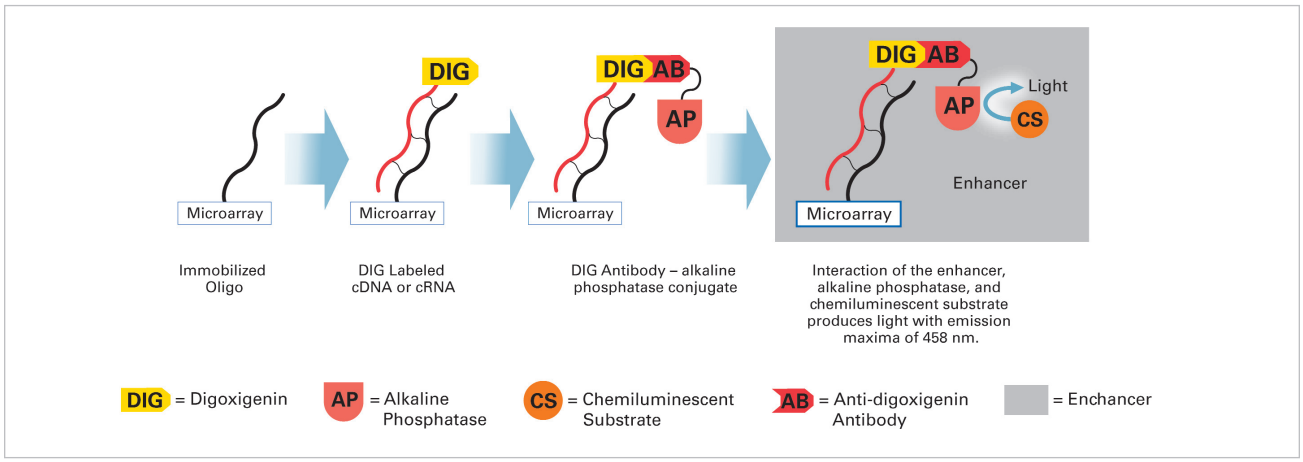


**Figure 4.** Schematic of the labeling and chemiluminescence detection process used in the Expression Array System Microarray assay

### Detection Kit Components

The Applied Biosystems Chemiluminescence Detection Kit contains all buffers and reagents required for hybridization, washing, and detection of chemiluminescent signal. The sole exception is the anti-digoxigenin/alkaline phosphatase conjugate, which is available from Roche Applied Science.

### Kit Controls

All three chemiluminescent kits incorporate a wide range of controls to ensure accurate microarray data analysis. These include controls for both the hybridization and labeling reactions. The controls provide data about RT and RT-IVT enzyme activity, DIG-label incorporation, the wash procedure, and the chemiluminescence detection chemistries.

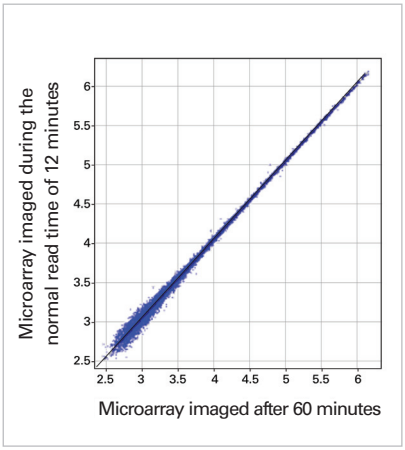


**Figure 5.** Scatter plot of chemiluminescent signals from the same microarray, imaged during the normal read time (~12 minutes) and after 60 minutes, using the Applied Biosystems 1700 Chemiluminescent Microarray Analyzer. During the first 5 minutes after the array is placed into the instrument, the microarray reaches 35°C and the light production reaches a steady state. The system detects > 16,000 genes after hybridization of labeled cRNA, derived from the Stratagene UHR to the Applied Biosystems microarray.

Additionally, the Chemiluminescence Detection Kit includes a 24-mer oligonucleotide labeled with the fluorescent LIZ® dye. The labeled oligonucleotide is complementary to a 24-mer probe that is co-deposited at every feature during the manufacture of Applied Biosystems microarrays, and therefore binds to every feature during hybridization.

Fluorescence imaging of the microarray enables visualization of every feature, completely independent of the chemiluminescent signal (gene expression). Fluorescence provides auto-gridding of the microarray, along with feature-to-feature and array-to-array signal normalization.

## References


[1] Van Gelder, R.N. et al., *Proc. Natl. Acad. Sci. USA*, 87: 1663–1667 (1990)

[2] Bronstein, I, Olesen, et al., *Bioluminescence and Chemiluminescence: Fundamentals and Applied Aspects*, 269–272 (1994)


## Ordering Information

| Description | Reactions | P/N |
|---|---|---|
| Applied Biosystems NanoAmp™ RT-IVT Labeling Kit<br>T7 Oligo (dT) Primer, Control RNA, 10X 1st Strand Buffer, RT Enzyme, 10X 2nd Strand Buffer, DNA Polymerase/RNase H/RNase Inhibitor, dNTP Mix, IVT Control DNA, 10X IVT Buffer, IVT Enzyme Mix, NTP Mix, 2nd Round Primers, Nuclease-free Water, Wash Buffer, RNA Binding Buffer, DNA Binding Buffer, RNA Purification Columns, DNA Purification Columns, RNA Collection Tube, DNA Elution Tubes | 12 | 4367515 |
| Applied Biosystems Chemiluminescent RT Labeling Kit<br>Poly dT Primer, 10X RT Buffer Mix, RT Enzyme Mix, NaOH, Tris, DNA Binding/Wash/Elution Buffers,ETOH, RT Bacterial Controls, and cDNA Purification Columns and Tubes | 12 | 4340415 |
| Applied Biosystems Chemiluminescence Detection Kit<br>Hybridization Buffer, Hybridization Controls,Denaturant, Hybridization Wash Concentrate, Detergent, Blocking Reagents, Chemiluminescent Substrate, Chemiluminescent Enhancer, Cover Slides, and Rinse Buffer | 12 | 4342142 |

| Additional Reagents* | Required for | Quantity |
|---|---|---|
| Dioxigenin-11-dUTP | RT Kit | 25 nmol/25 µL |
| Dioxigenin-11-uridine-5′-triphosphate | NanoAmp™ RT-IVT Kit | 200 nmol/57 µL |
| Anti-digoxigenin-alkaline phosphatase | Detection Kit | 150 U/200 µL |

*These products, which are required but not included, are available from Roche Applied Science.




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