WHAT IS LABELING?

A wide variety of molecular and cellular biology procedures are dependent on a labeled or tagged nucleic acid whose behavior can be specifically studied via the attached label. Ideally, the label will have little to no influence on the behavior of the labeled nucleic acid. There are different ways to classify nucleic acid labeling methods:

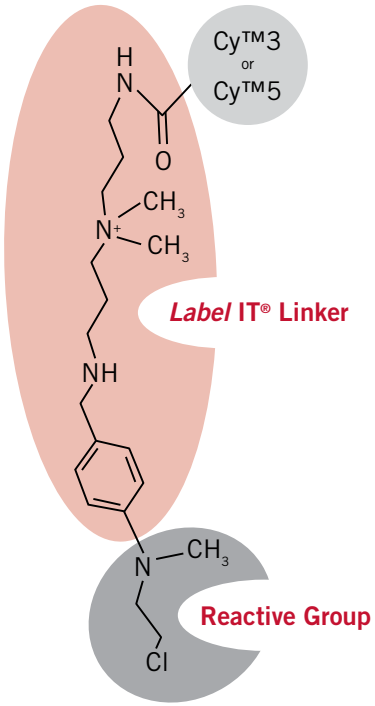
Chemical labeling technologies are based on reactive groups that bind to nucleic acids. Labeling reagents react with the nucleic acid and chemically attach labels to it. For example, the *Label* IT labeling reagents (see schematic diagram) consists of the label, a cationic linker and an alkylating reactive group. These reactions do not require enzymes.

Enzymatic labeling protocols are based on the incorporation of label-modified nucleotides during the enzyme-mediated nucleic acid synthesis reaction.

Labeled nucleic acids can be detected in different ways:

Direct detection—the labeled nucleic acids contain the detector molecule that will be used for optical, luminescent or fluorescent signal generation.

Indirect detection—the labeled nucleic acids contain a label or tag which needs to be specifically bound by a reporter-conjugated affinity molecule (for example, biotin with a fluorophore-conjugated streptavidin, or digoxin with a fluorophore-conjugated specific antibody).



Fluorophore and Nucleic Acid Spectral Characteristics

Fluorophore	Excitation Wavelength or l_{MAX} (nm)	Emission Wavelength (nm)	Extinction Coefficient (e_{dye}, M^{-1} cm^{-1})	Correction Factor ($C.F._{260}$)
Cy™3	550	570	150,000	0.08
Cy™5	649	670	250,000	0.05
CX-Rhodamine	576	597	82,000	Not determined
TM-Rhodamine	546	576	100,000	0.27
Fluorescein	494	518	30,000	0.32

Nucleic acid	Extinction Coefficient (e_{base}, M^{-1} cm^{-1})
dsDNA	6,600
ssDNA	8,919
oligonucleotide	10,000
RNA	8,250

How to Determine Labeling Density

The most straight-forward way to estimate the density of fluorophores on the labeled and purified DNA (or RNA) sample involves measuring the absorbance of the nucleic acid-dye conjugate at 260 nm (A_{260}) and the I_{MAX} for the particular dye (A_{dye}).

For most applications, the absorbance of the entire sample, using a spectrophotometer with a microcell, may be required to generate reliable absorbance readings. If samples are going to be quantified spectrophotometrically, we recommend purifying the labeled nucleic acid by ethanol precipitation or a silica membrane-based column. While gel filtration is an effective purification method, we observe erroneously high spectrophotometer readings (at A_{260}) when using this method and it is therefore not recommended.

Estimating labeling density (2 methods; refer to the table, "Fluorophore and Nucleic Acid Spectral Characteristics"):

1. Base: Dye Ratio

Step 1. Calculate A_{base} (correct the A_{260} reading for any contribution from the dye in the sample) using:
$A_{base} = A_{260} - (A_{dye} \times C.F._{260})$

Step 2. Calculate the ratio of bases to dye molecules:
base:dye = $(A_{base} \times e_{dye}) / (A_{dye} \times e_{base})$

2. Concentration (pmol of dye per µg of nucleic acid)

Step 1. Determine the dye concentration ([dye], mol/liter), by calculating A_{dye}/e_{dye}

Step 2. Calculate pmol dye in sample, using the following equation:
pmol dye = [dye] $\times 10^{12}$ pmol/mol $\times$ sample volume (in liters)

Step 3. Calculate amount of recovered nucleic acid:
µg DNA = $A_{base} \times 50$ µg/ml (for ds DNA) $\times$ sample volume (in ml) (Use 40 µg/ml for RNA)

Step 4. Determine sample concentration by dividing pmol dye (from step 2) by µg nucleic acid (from step 3).

Altering Labeling Reaction/Density

Different research applications may require different nucleic acid label densities for optimal performance. For example, in nucleic acid tracking experiments, both *in vitro* and *in vivo*, optimal fluorescence detection can be achieved with highly labeled nucleic acids. However, in tracking experiments where the biological functionality of the labeled nucleic acid (*e.g.* plasmid) is also desired, a lower labeling density will be required. With the transfection of highly labeled plasmids, transcription will likely be inhibited, compared to unlabeled plasmids. With the *Label* IT technology, it is possible to adjust the density of labels on the nucleic acid, the volume of the labeling reaction and the amount of nucleic acid in the labeling reaction.

To modify the labeling density, **vary the ratio of the labeling reagent to nucleic acid** during the labeling reaction or **adjust the incubation time** of the labeling reaction. With the general *Label* IT Nucleic Acid Labeling Kits (refer to page 26, we observe a linear increase in labeling density during the first 3 hours of incubation at 37°C. We recommend a standard reaction for 1 hour at 37°C. Generally, twice the labeling density will be achieved if the duration of the labeling reaction is extended to 2 hours at 37°C. Also, with the general *Label* IT Nucleic Acid Labeling Kits, modest

changes (2–4 fold) in the ratio of *Label* IT Reagent to nucleic acid affect the labeling efficiency in a linear manner. Dramatic increases to the recommended 1:1 (µl *Label* IT Reagent:µg DNA) ratio will result in increased labeling, but not necessarily of a linear nature. Dramatic increases to the recommended labeling ratio may also increase the chance of nicking the nucleic acid template.

In addition, the volume of the labeling reaction may be scaled up or down without affecting the labeling performance. In such reactions, ensure that the amount of *Label* IT Reagent does not consist of more than 20% of the total reaction volume and a final concentration of Labeling Buffer A is maintained at 1X in the reaction.

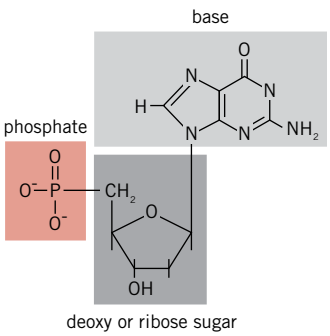
Inhibitors of Labeling

The *Label* IT Labeling Reagent comprises a reactive alkylating agent with strong nucleic acid binding capability facilitated via electrostatic interactions. For the best and most consistent labeling performance, use clean, intact nucleic acid in your labeling reactions. Dirty nucleic acid preparations may compromise the efficiency of labeling reactions—due to the presence of contaminants which affect (or compete with) the electrostatic interaction between the reagent and nucleic acid. **Common inhibitors include: salts, bacterial endotoxin, and proteins**.

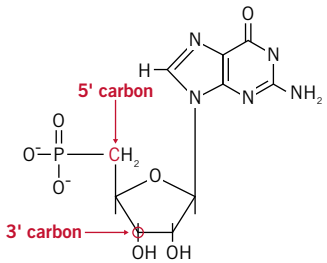
Microarray Schematics and Illustration of Polarity Issues

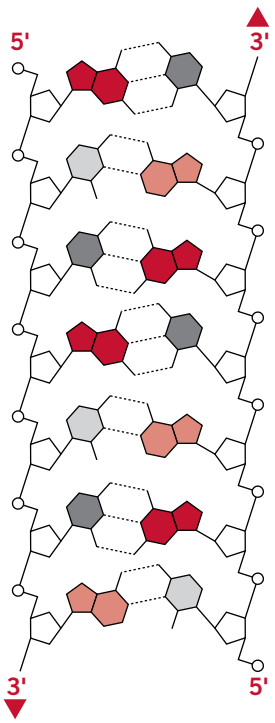
Single-stranded nucleic acid polymers have a polarity, or directionality, defined by the presence of the unpolymerized 5' carbon at one end of the molecule, which most often exists as a 5'-phosphate, and the unpolymerized 3' carbon at the other end of the molecule, which most often exists as a 3'-hydroxyl residue. With double-stranded nucleic acids, the two strands are arranged antiparallel to one another—one strand is 5' to 3', while the other strand is aligned 3' to 5'. A DNA segment encoding a protein usually has a "sense" strand and a complementary "antisense" strand which acts as a template for RNA polymerase. Conventionally, the sense strand is considered to encode the protein since it has the same sequence as the mRNA.

Deoxyguanosine monophosphate



(ribo) guanosine monophosphate





In hybridization studies, it is important to understand the polarity of the nucleic acid samples as the probe and target must be complementary (or basepair) for hybridization to occur. As you will note in the illustration, this is particularly important in microarray experiments, since a variety of labeled nucleic acids can be generated from an RNA sample and different printed capture sequences are available.