DNA Labeling Kits Fluorescein-dCTP and -dUTP

Instruction Manual

Catalog Number

170-8223
170-8224

For Technical Service
Call Your Local Bio-Rad Office or
in the U.S. Call **1-800-4BIORAD**
(1-800-424-6723)

BIO-RAD

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4100122 Rev A

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Section 1
Introduction

This manual contains background information and a detailed protocol for end-labeling DNA with a fluorescein tagged nucleotide. This simple technique lets you visualize DNA samples without additional staining. Fluorescent dye conjugates have been used in many different molecular biology applications, for example, DNA sequencing and mapping. These kits will enzymatically end-label a variety of double-stranded DNA samples. The end-labeling is especially effective in multiplexed electrophoresis, where fluorescein-labeled samples are run in combination with Texas Red® DNA size standards, and provides the highest possible accuracy of molecular weight estimations.

Consistent with the widespread desire to reduce hazardous waste, fluorescent end-labeling eliminates the need to stain gels with ethidium bromide, or to use radioactive tags. The staining and destaining procedure is the time equivalent to that of the end-labeling reaction. The reaction does not require specialized equipment.

The kit is available in two versions. One contains a fluorescein-dUTP, and the other a fluorescein-dCTP. The kit utilizes the properties of T4 DNA polymerase[1] to incorporate a fluorescent dye-tagged nucleotide into dsDNA. Because there is a choice of labeled nucleotide, the two kits can be used to label most dsDNA samples. To select the appropriate kit configuration, identify a 5 prime end with

an available A or G, and label with a complementary fluorescein-dUTP or -dCTP respectively. For samples of unknown sequence, we recommend that you try both reactions, one of each tagged nucleotide, since one may give a better result than the other.

Bio-Rad's kit provides high quality reagents and all components are subjected to stringent quality control procedures to insure success in labeling reactions. If you have any questions regarding the use of this product, contact your local Bio-Rad representative, or, in the U.S., call 1-800-4BIORAD.

Section 2
Kit Components

2.1 Kit Description

Fluorescein-dCTP Kit

T4 DNA Polymerase	65 units	13 µl
Fluorescein-dCTP	10 nMoles	10 µl
Reaction Buffer (with dATP, dTTP, dGTP)	10x	120 µl
Control DNA	0.2 µg/µl	20 µl
Loading Buffer	5x	500 µl

Fluorescein-dUTP Kit

T4 DNA Polymerase	65 units	13 µl
Fluorescein-dUTP	10 nMoles	10 µl
Reaction Buffer (with dATP, dCTP, dGTP)	10x	120 µl
Control DNA	0.2 µg/µl	20 µl
Loading Buffer	5x	500 µl

Each kit has sufficient materials to label 50 µg of dsDNA sample.

Both kit configurations contain Bio-Rad T4 DNA polymerase, and the components are optimized for this enzyme. Other T4 DNA polymerases may be substituted, but the labeling may not be as efficient. Store the enzyme at -20 °C.

Fluorescein nucleotide comes in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.9) and is best stored at -20 °C. It can be diluted 1 to 10 in TE for convenient pipetting. Be sure to reduce the water in the reaction accordingly.

The reaction buffer is composed of: 10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, and 1 mM DTT at pH 7.9, and contains free 10 mM dNTPs other than the dye-labeled nucleotide. Store at -20 °C.

The Control DNA is an unlabeled restriction digest of pBR322 plasmid, prepared using Ava II and EcoR I. This allows you to test the labeling reaction on a known DNA sequence. The fragments are labeled differently when using dUTP or dCTP (see Figure 1). Store at 4 °C for the short term and -20 °C for the long term.

The loading buffer was designed for use with fluorescent DNA samples, and contains only bromophenol blue; xylene cyanol interferes with the visualization of bands that co-migrate with this indicator dye. Store at 4 °C for the short term and -20 °C for the long term.

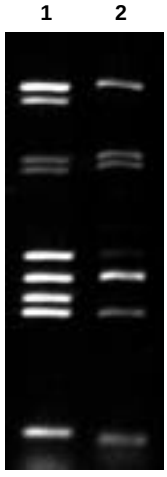

Fig. 1. Control DNA gel. Lane 1 contains the bands of the Control DNA labeled with the dCTP kit. Lane 2 contains the bands of the Control DNA labeled with the dUTP kit. The DNA was run on a 5% TBE Ready Gel at 100 V for 50 minutes.

2.2 Related Products

Catalog Number	Product Description
170-8221	**DNA Labeling Kit, Texas Red-dCTP**
170-8222	**DNA Labeling Kit, Texas Red-dUTP**
170-3123	**Fluorescein Low Range DNA Standard**
170-3124	**Texas Red Low Range DNA Standard**
170-8216	**100 bp Fluorescein Molecular Ruler**
170-8217	**100 bp Texas Red Molecular Ruler**
170-8218	**500 bp Fluorescein Molecular Ruler**
170-8219	**500 bp Texas Red Molecular Ruler**

Section 3
Applications

3.1 Typical End-Labeling Experiments

For achieving optimum results, knowledge of the end sequence of the DNA target is helpful, but not mandatory. The labeling reaction is carried out by T4 DNA polymerase. The T4 DNA polymerase has two enzymatic activities first to fill in under a 5'- overhang in the presence of excess free nucleotides, and second, a very strong 3'- to 5'- exonuclease activity which makes possible the labeling of blunt ends and PCR fragments by an exchange reaction.

Figure 2 is an example of a 5'- overhang, which is best labeled with a fill-in reaction. Digestion by the restriction enzyme BamH I leaves a 5'- overhang which may be labeled with either a dUTP or a dCTP kit.

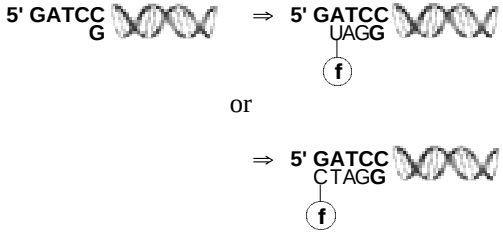

Fig. 2. 5' overhang.

In general, when an adenosine is exposed, use dUTP, and when a guanosine is exposed, use dCTP; both work equally well in the example above. A free dCTP may or may not be incorporated following addition of the labeled uracil, but this addition it is not necessary for a good result. Generally one dye labeled nucleotide is incorporated in any end, although multiple incorporation is theoretically possible.

A 3'- overhang requires a removal and replacement reaction. The restriction enzyme Pst I leaves a 3'- tail as shown in Figure 3.

Fig. 3. 3' overhang.

The 3'- to 5'- exonuclease activity of T4 DNA polymerase removes the 3'- tail plus an additional unknown number of nucleotides leaving a 5'- overhang. The end may then be labeled using the appropriate nucleotide.

Blunt ends may be labeled in the same manner as 3'- overhangs. Using the protocol provided in Section 4, labeling is achieved by removal of the 3'- bases. The actual number of bases removed cannot be determined or controlled, but experiments have been successful with removal of up to as many as eight bases underneath the 5'- overhang.

PCR* products can be labeled with some success, but not all PCR products label with the same efficiency. Ethanol precipitation can help the reaction by removing the excess nucleotides. If labeling a product directly after PCR, it is best to increase the units of enzyme to 3 units per microgram of product. Additional T4 DNA polymerase can be purchased as a separate item. Knowledge of the primers will help to determine the necessary nucleotide to use for label. The best results occur when the A or G is close to the 5' end.

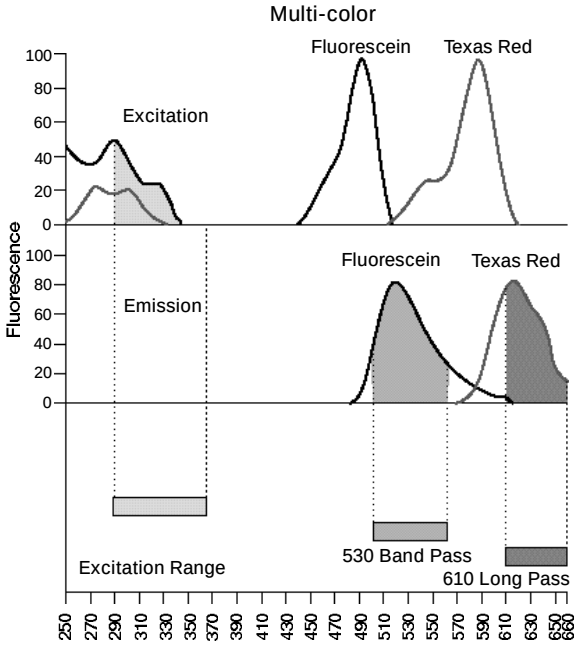

Fig. 4. Excitation and detection of multi-color on the Fluor-S MultiImager system.

3.2 Visualization Methods

Fluorescein has an excitation peak which is excitable with a 302 nm UV light source, and a visible excitation peak excitable with a 488 nm argon laser. The emission peak is at 520 nm. When visualizing fluorescein on the Fluor-S™ MultiImager system, use the 530 band pass filter. Figure 4 depicts the absorption and emission spectra for Texas Red and for fluorescein. Fluorescein is a dye that can be multiplexed with Texas Red on the Fluor-S MultiImager system. End-labeling with a fluorescent tagged nucleotide minimally affects migration of the DNA sample.

Section 4
Protocol

The following protocol can be scaled depending on the amount of dsDNA to be labeled. The reaction for 1 µg of dsDNA is shown here to simplify the scaling. The reaction for Fluorescein-dCTP is the same as Fluorescein-dUTP shown below.

Optimum labeling occurs when the final concentration of DNA is 0.05 µg/ µl. The reaction buffer is a 10x buffer, therefore 10% of the reaction volume. The Fluorescein dUTP can be diluted 1 to 10. If this is done, use 2 µl instead of 0.2 µl. Be sure to adjust the amount of water added. Diluting the enzyme is not recommended. When combining the components, add the enzyme last. DNA is normally in TE buffer, and the conditions of this reaction were optimized for DNA

in TE buffer. Other buffers will not, in most cases, adversely affect the reaction. For example, a restriction digest generally does not need to be cleaned up before using this reaction.

Combine the following components, in a microfuge tube, vortex, and spin briefly.

DNA 1 µg at 0.1 µg/µl	10.0 µl
ddH_2O (to 20 µl total volume)	7.6 µl
Reaction buffer with nucleotides	2.0 µl
Fluorescein-dUTP	0.2 µl
T4 DNA polymerase	0.2 µl
Total volume	20.0 µl

1. Incubate at room temperature (20 to 25 °C) for 20–30 minutes.
2. Ethanol precipitate by adding 2 µl of 10 M ammonium acetate (10% volume) and 55 µl of 95% ethanol (2.5x volume).
3. Put tubes on ice for 10 to 15 minutes.
4. Centrifuge at 14,000 rpm in a microcentrifuge for 20 minutes, then remove immediately, pour off supernatant, and invert.
5. Rinse twice with 55 µl of 70% ethanol, pouring off the ethanol each time. Rinse once with 55 µl of 100% ethanol, remove all ethanol, and dry pellets. Pellets of less than 10 µg will most likely be invisible.
6. Resuspend in TE (10 µl for a concentration of 0.1 µg/µl). Vortex and spin.

7. Use 4 µl of the resuspended sample with 1 µl of 5x loading buffer, and load on an agarose or acrylamide gel.

When using the Control DNA, use

Control DNA (0.2 µg/µl)	5.0 µl
ddH_2O	12.6 µl
Reaction Buffer, dUTP kit	2.0 µl
Fluorescein dUTP	0.2 µl
T4 DNA polymerase	0.2 µl
Total volume	20.0 µl

Ethanol precipitate and visualize along with samples.

Section 5 Troubleshooting

The labeling reaction is usually very efficient, but some problems may occur. Here is a list of typical problems.

Problem	**Cause**
Excess free dye visualized	Incomplete ethanol rinsing
Sample floats out of the well	Incomplete drying of the pellet
No labeling	Check for compatible sequence and that all components were added.
Non uniform bands	Incomplete mixing of sample with loading buffer

Section 6
References

1. Maniatis, T., Fritsch, E. F., and Sambrook, J. Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1982).