ProductInformation

BioProbe®
Random Primed DNA Labeling System

Reagent Pack
[25 labeling reactions]

Cat. No. B-7548

For use with Random Primed DNA Labeling System Deoxynucleotide Packs:

Bio-11-dUTP	**Cat. No. B-7298**
Bio-16-dUTP	**Cat. No. B-6673**
Bio-11-dCTP	**Cat. No. B-6548**
Bio-7-dATP	**Cat. No. B-6423**
Fluorescein-12-dUTP	**Cat. No. B-7423**

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INTRODUCTION

Labeled nucleotides can be incorporated into double stranded or single stranded DNA using a random primed synthesis reaction. This labeling method is based on the annealing of random sequence hexanucleotide primers to a denatured DNA template (Feinberg, A.P. and Vogelstein, B. [1983] Anal. Biochem. **132**, 6). The complementary strand is then synthesized by Klenow fragment of DNA polymerase I by addition of nucleotide residues to the 3'-OH terminus of the hexanucleotide primers, resulting in a net synthesis of DNA. Inclusion of modified nucleotide analogs in the synthetic reactions results in chemically modified DNA. In one hour to overnight reactions, newly synthesized labeled DNA is generated in high yield.

Random primed labeling reactions are optimized for generating maximum amounts of labeled DNA from 50 ng to 1 µg of DNA template. The reactions can be performed with denatured DNA derived from complete plasmid DNA or from gel-isolated restriction fragments of DNA. The reaction products range in size from less than 200 to greater than 2000 base pairs, depending upon the starting DNA fragment size.

Random primed probes are excellent for use in membrane hybridization applications and can be used in many circumstances for *in situ* hybridization studies. Standard hybridization and detection methods are used with chemically modified, random primed DNA probes.

A complete ***BioProbe***® **Random Primed DNA Labeling System** consists of the combination of two separate components: a Reagent Pack and one of five different Deoxynucleotide Packs. The ***BioProbe***® **Random Primed DNA Labeling System Reagent Pack** contains all of the reagents (except deoxynucleotides) required to carry out 25 nonradioactive random primed DNA labeling reactions, including 10X hexanucleotide primers and Klenow DNA Polymerase. The Reagent Pack is completed by a vial of Stop Buffer and a vial of Control Template DNA. The various ***BioProbe***® **Random Primed DNA Labeling System Deoxynucleotide Packs** contain the modified deoxynucleotide mixed with the other deoxynucleoside triphosphates at an optimized concentration. A vial of Control DNA labeled with the analog nucleotide contained in the specific deoxynucleotide mixture completes the deoxynucleotide pack.

The methods for labeling DNA with the ***BioProbe***® **Random Primed DNA Labeling System** are consistent for all of the haptens. Labeling reactions using different analog nucleotides and different DNAs can be carried out at the same time with the same reaction conditions. Thus, combination of the Reagent Pack with two different Deoxynucleotide Packs provides a simple, convenient and economical way to label DNA probes with two different haptens.

REAGENTS PROVIDED

10X Hexanucleotide Primers, 125 µl
10X concentrated in 10X Reaction Buffer

Klenow DNA Polymerase , 50 µl
2 units/µl in storage buffer

Stop Buffer, 125 µl
0.2 M EDTA

Control Template DNA, 25 µl
10 ng/µl in TE Buffer (10mM Tris Buffer, pH 8.0, 1mM EDTA)

ADDITIONAL REAGENTS REQUIRED
(Available separately or combined with Cat. No. B-7548)

Random Primed DNA Labeling Deoxynucleotide Pack:
- Deoxynucleotide Mix, 125 µl
  10X concentrated mixture of dATP, dCTP, dGTP, TTP and modified dNTP in Tris buffer
- Labeled Control DNA, 25 µl
  Comprised of 50 ng of Control Template DNA plus the newly synthesized labeled DNA from a standard 2 hour reaction

EQUIPMENT AND REAGENTS REQUIRED BUT NOT PROVIDED

Preparation and Analysis of Random Primed Probes
- 37°C Water Bath
- Agarose
- Ethidium Bromide
- UV Transilluminator

Purification of Random Primed DNA Probes
- 4M LiCl
- Prechilled (-20°C) Ethanol
- TE Buffer
- Microcentrifuge

STORAGE
1. Upon receipt, store all reagents at -20°C, in a freezer that is not self defrosting.
2. After initial use, continue to store reagents at -20°C.

RANDOM PRIMED LABELING OF DNA

A. Template DNA Preparation

DNA in Solution

1. Denature the DNA by boiling for 10 minutes and then quick chill in ice. The volume of template DNA solution should not exceed 38 µl per 50 µl reaction.

   For a control reaction use 5 µl of Control Template DNA.

DNA Fragments in Low Melting Point Agarose

1. From a low melting point agarose gel, cut out the DNA fragment to be labeled with a minimum of excess gel. Transfer the gel slice into a pre-weighed microcentrifuge tube. Determine the weight of the gel slice.
2. Add 3 ml of deionized water per gram of gel and boil the tube for 10 minutes to melt the gel and denature the DNA.
3. Cool the DNA/agarose mixture to 37°C. The DNA is now ready for labeling. Up to 38 µl of this material may be used in a labeling reaction.

B. Standard Labeling Procedure

1. To a clean microcentrifuge tube on ice add the following in the order shown.

Reagent	Volume
Deoxynucleotide Mix (From Random Primed DNA Labeling Deoxynucleotide Pack)	5 µl
10X Hexanucleotide Primers	5 µl
Denatured template DNA	variable* (50 ng-1 µg)
Distilled or deionized water	to 48 µl*
Klenow DNA Polymerase	2 µl
Total Volume	**50 µl**

**The combined volume of template DNA and water must not exceed 38 µl.*

2. Mix the reagents in the tube and collect the mixture in the bottom of the tube by brief (5 second) microcentrifugation.
3. Incubate the tube for at least I hour in a 37°C water bath. Longer incubation (up to 20 hours) can increase the yield of labeled DNA.
4. Add 2 µl of Stop Buffer to terminate the reaction.

LABELING EFFICIENCY

The amount of newly synthesized labeled DNA is dependent on the amount and purity of template DNA, the length of incubation time at 37°C and the type of modified nucleotide used for labeling. Reaction with less template DNA can result in a greater ratio of labeled DNA to template DNA, as seen in the following example using the Bio-16-dUTP label.

Amount of template Lambda DNA per 50 µl reaction	50 ng	200 ng	1000 ng
Amount of newly synthesized Bio-16-dUTP labeled DNA			
after 1 hour	175 ng	590 ng	1340 ng
after 2 hours	300 ng	950 ng	1550 ng
after 5 hours	500 ng		

DNA synthesis was measured by incorporation of ^{3}H-dNTP

PURIFICATION OF BIOTIN and FLUORESCEIN LABELED DNA PROBES

For most membrane applications the labeled DNA can be used without further manipulation because unincorporated nucleotides do not adhere to the membrane and are washed away during detection. If purification is desired, the following protocol may be used.

1. Add 10 µl of 4M LiCl and 200 µl of prechilled (-20°C) ethanol. Mix well.
2. Leave for 30 minutes at -70°C or 2 hours at -20°C.
3. Centrifuge at high speed (13,000 x *g*) for 10-15 minutes.
4. Remove and discard supernatant, being careful not to disturb the pellet.
5. Wash the pellet with 100 µl of prechillled (-20°C) ethanol, vortex to mix and then centrifuge for 10-15 minutes.
6. Remove and discard supernatant, being careful not to disturb the pellet.
7. Dry the DNA pellet briefly under vacuum and dissolve in small volume of TE buffer to the desired concentration.
8. Store the labeled probe at -20°C or -70°C. Probes are stable for several years when stored frozen.

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For Technical Assistance
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