BioPrime® DNA Labeling System

Catalog. No.: 18094-011

Size: 30 Reactions
Store at -20°C
in a non-frost-free freezer.

Description

The BioPrime® DNA Labeling System is specifically designed for use in the preparation of biotinylated probes. Random primers (octamers) are annealed to the denatured DNA template and extended by Klenow fragment in the presence of biotin-14-dCTP to produce sensitive biotinylated-DNA probes for use in the nonradioactive detection of DNA and RNA. With the BioPrime® DNA Labeling System, considerable net DNA synthesis occurs resulting in a 10-40 fold amplification of the probe. This product is particularly well suited for situations in which the DNA is limited. Probes made with this product have been used for hybridization to Southern and Northern blots, plaque and colony lifts, and for *in situ* hybridization to chromosome spreads.

Components	Part No.	Amount
2.5X Random Primers Solution: [125 mM Tris-HCl (pH 6.8), 12.5 mM $MgCl_2$, 25 mM 2-mercaptoethanol, 750 µg/ml oligodeoxyribonucleotide primers (random octamers)]	Y01393	700 µl
10X dNTP Mixture: [1 mM biotin-14-dCTP, 1 mM dCTP, 2 mM dATP, 2 mM dGTP, 2 mM dTTP in 10 mM Tris-HCl (pH 7.5), 1 mM Na_2EDTA]	Y01394	175 µ l
Control DNA: [Salmon Sperm DNA, 10 µg/µl in RNAse-free, DNAse-free water]	55302	100 µg
Klenow Fragment (Large Fragment of DNA Polymerase I): [40 U/µl Klenow Fragment in 50 mM Potassium Phosphate (pH 7.0), 100 mM KCl, 1 mM DTT, 50% Glycerol]	Y01396	35 µl
Stop Buffer: [0.5 M Na_2EDTA (pH 8.0)]	50690	500 µl
Distilled Water	50837	1.25 ml

BioPrime® Labeling Protocol

1. Dissolve 100 ng DNA in 5-20 µl of dilute buffer in a microcentrifuge tube. On ice, add 20 µl 2.5X Random Primers Solution. Denature by heating for 5 min in a boiling water bath; immediately cool on ice. (The amount of template per reaction has been varied from 25-500 ng with satisfactory results.)
2. Perform the following additions on ice:

   5 µl 10X dNTP Mixture
   Distilled Water to a total volume of 49 µl
3. Mix briefly.
4. Add 1 µl Klenow Fragment. Mix gently but thoroughly. Centrifuge 15-30 sec.
5. Incubate at 37°C for 60 min.
6. Add 5 µl Stop Buffer.

7. Unincorporated nucleotides can be separated from biotinylated-DNA probe by repeated ethanol precipitation. This procedure produces satisfactory probes for filter hybridizations.

   Repeated ethanol precipitation: Add 1/10 volume 3 M sodium acetate and two volumes cold 95% (or absolute) ethanol to the reaction tube. Mix by inverting the tube. Freeze at -70°C (dry ice) for 15 min or at -20°C for 2 h. Centrifuge at 15,000 × *g* for 10 min. Carefully remove the supernatant with a pipette and dry the pellet. Resuspend the probe in 50 µl distilled water and precipitate the probe with sodium acetate and ethanol as described above. Resuspend the probe in TE buffer [10 mM Tris-HCl (pH 7.5), 1 mM Na_2EDTA] and store at -20°C.

   PureLink™ PCR Purification System (Cat. Series K3100): Follow instructions provided with the kit.

8. Biotinylated probes are very stable and can be stored at -20°C for at least one year.

Notes

1. Net DNA synthesis occurs during random-primer labeling reactions. Using 100 ng of Salmon Sperm DNA, the amount of biotinylated probe DNA synthesis is greater than 1 µg, as measured by a UV/visible spectrophotometer. Amplification of probe with 100 ng template routinely varies from 10- to 40-fold in a 1-h reaction.
2. Difficulties in random-primer labeling of DNA often result from contaminants in the template DNA preparation. To a certain extent these problems may be overcome by extending the reaction time to 2-4 h and/or by increasing the amount of enzyme to 2 µl per reaction.

References

1. Feinberg, A. P. and Vogelstein, B. (1983) *Anal Biochem 132*, 6.
2. Feinberg, A. P. and Vogelstein, B. (1984) *Anal Biochem 137*, 266.