Invitrogen
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RadPrime DNA Labeling System

Cat. No.: 18428-011 **Size: 30 Reactions**

Store at -20°C in a non-frost-free freezer.

Description:

The RadPrime DNA Labeling System is designed for rapid preparation of high specific activity ^{32}P-labeled probes. Random primers (octamers) are annealed to the denatured DNA template and extended by Klenow fragment in the presence of an α–^{32}P-dNTP to produce sensitive high-specific activity DNA probes for use in detection of DNA and RNA. Labeling is complete in 10 minutes; purification of the probe is not usually required.

Components:	Part No.	Amount
2.5X RadPrime Buffer: 125 mM Tris-HCl (pH 6.8); 12.5 mM magnesium chloride; 25 mM 2-mercaptoethanol; 150 µg/ml oligodeoxyribonucleotide primers (random octamers)	Y02360	700 µl
dATP Solution: 500 µM in 1 mM Tris-HCl (pH 7.5)	Y01102	100 µl
dCTP Solution: 500 µM in 1 mM Tris-HCl (pH 7.5)	Y01103	100 µl
dGTP Solution: 500 µM in 1 mM Tris-HCl (pH 7.5)	Y01104	100 µl
dTTP Solution: 500 µM in 1 mM Tris-HCl (pH 7.5)	Y01105	100 µl
Klenow Fragment (Large Fragment of DNA Polymerase I): 40 U/µl Klenow fragment in 50 mM potassium phosphate buffer (pH 7.0), 100 mM KCl, 1 mM DTT, 50% (v/v) glycerol	Y01396	35 µl
Control DNA: 5 ng/µl pBR322 DNA/*Rsa* I fragments in 10 mM Tris-HCl (pH 7.4), 5 mM NaCl, 0.1 mM EDTA.	50981	30 µl
Stop Buffer: 0.5 M EDTA (pH 8.0).	50690	500 µl
Distilled Water:	50837	1.25 ml

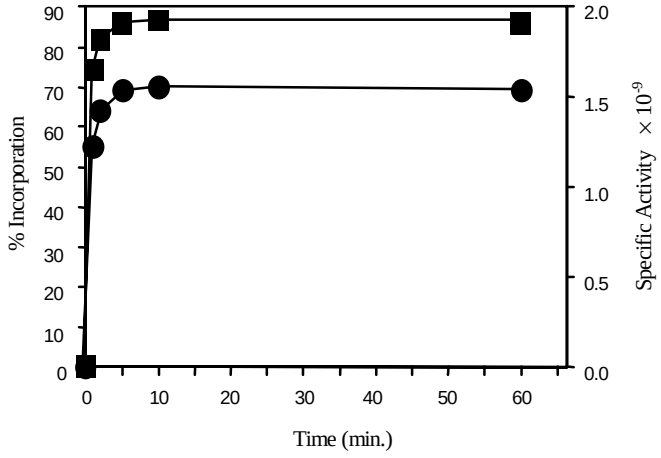

Figure 1. Kinetics of RadPrime System.

When 25 ng of control DNA was labeled using the RadPrime DNA Labeling System, a specific activity of $>1 \times 10^9$ cpm/µg DNA was obtained in 10 min or less at 37°C. Typical results are shown above. Circles: Percent Incorporation of ^{32}P-dNTP. Squares: Specific Activity of DNA in cpm/µg.

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Standard Labeling Protocol:

1. Denature 25 ng DNA dissolved in 5-20 µl of sterile distilled water or TE in a microcentrifuge tube by heating for 5 min in a boiling water bath; then immediately cool on ice.

2. Perform the following additions on ice:

   1 µl 500 µM dATP
   1 µl 500 µM dGTP
   1 µl 500 µM dTTP
   20 µl 2.5X Random Primers Solution
   5 µl (approximately 50 µCi) [α-^{32}P]dCTP, 3000 Ci/mmol, 10 mCi/ml
   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 s.

5. Incubate at 37°C for 10 min.

6. Add 5 µl Stop Buffer.

7. Dilute 2 µl of the mixture with 98 µl TE buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA].

8. Spot 5 µl of this dilution on a glass fiber filter (Whatman GF/C or equivalent). Wash the filter three times with 50 ml of cold 10% (w/v) TCA containing 1% (w/v) sodium pyrophosphate and once with 50 ml of 95% ethanol at room temperature.

9. Dry the filter under a heat lamp, and determine the precipitable radioactivity by liquid scintillation counting. Multiply the cpm obtained by 500 to determine the total cpm incorporated in the entire reaction mixture.

Notes:

1. The RadPrime DNA Labeling System contains the four deoxynucleoside triphosphates in four separate solutions. This allows the system to be used with any of the four [α-^{32}P]-labeled deoxynucleoside triphosphates by modifying the above protocol, which is given for [α-^{32}P] dCTP. The user may also elect to mix three of the nucleoside triphosphates in a cocktail in a 1:1:1 ratio, if a particular protocol is to be used consistently.

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 30 min and/or by increasing the amount of enzyme to 2 µl per reaction.

3. Increasing the amount of template DNA in the reaction will decrease the specific activity of the probe, while decreasing the template DNA will increase specific activity of the probe.

4. Generally, unincorporated nucleotides do not need to be separated from labeled DNA probe.

   If purification is desired, repeated ethanol precipitation may be used. 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 EDTA] and store at -20°C.

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.