GIBCOBRL

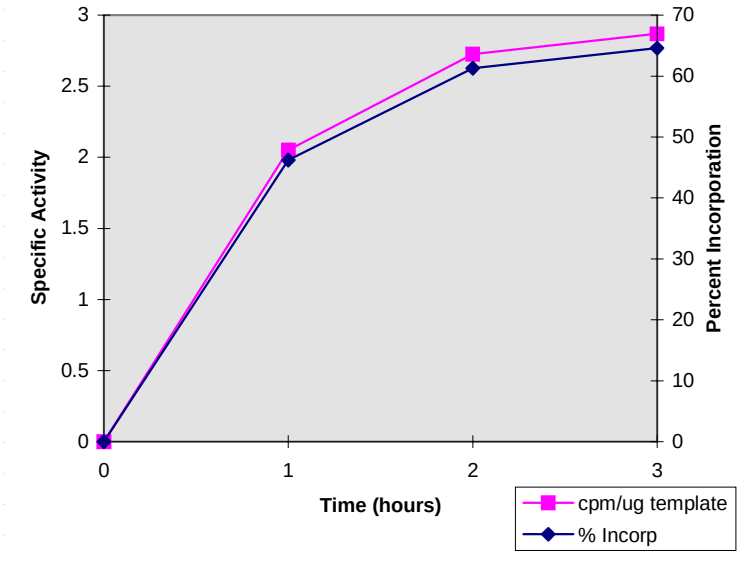
LIFE TECHNOLOGIES™

Random Primers DNA Labeling System

Cat. No.: 18187-013 **Size: 30 Assays**
Lot No.: **Store at -20°C in a non-frost-free freezer**

COMPONENTS:	PART NO.	AMOUNT
Random Primers Buffer Mixture: [0.67 M HEPES, 0.17 M Tris-HCl, 17 mM $MgCl_2$, 33 mM 2-mercaptoethanol, 1.33 mg/ml BSA, 18 OD_{260} units/ml oligodeoxyribonucleotide primers (hexamers), pH 6.8]	Y01101	500 µl
dATP Solution: 0.5 mM dATP in 1 mM Tris-HCl (pH 7.5)	Y01102	100 µl
dCTP Solution: 0.5 mM dCTP in 1 mM Tris-HCl (pH 7.5)	Y01103	100 µl
dGTP Solution: 0.5 mM dGTP in 1 mM Tris-HCl (pH 7.5)	Y01104	100 µl
dTTP Solution: 0.5 mM dTTP in 1 mM Tris-HCl (pH 7.5)	Y01105	100 µl
Klenow Fragment (Large Fragment of DNA Polymerase I): 3 U/µl Klenow fragment in 50 mM potassium phosphate buffer (pH 7.0), 100 mM KCl, 1 mM DTT, 50% (v/v) glycerol	Y01106	40 µl
Stop Buffer: 0.5 M EDTA, pH 8.0	50690	500 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
Distilled Water:	50837	1.25 ml

QUALITY CONTROL:
When 25 ng of the control DNA provided in this system is labeled using the standard Random Primers labeling conditions, a specific activity $\geq 1 \times 10^9$ cpm per µg of template DNA was obtained within 1 h at 25°C. Typical results are shown at right.



Doc. Rev.: 090398

STANDARD LABELING PROTOCOL:

The following instructions are given for a standard assay using 25 ng of DNA.

1. Denature 25 ng of DNA dissolved in 5-20 µl of distilled water 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:

   2 µl dATP solution
   2 µl dGTP solution
   2 µl dTTP solution
   15 µl Random Primers Buffer Mixture
   5 µl (approximately 50 µCi) [α-^{32}P]dCTP, 3000 Ci/mmol, 10 µCi/µl
   Distilled water to a total volume of 49 µl

   Mix briefly.

3. Add 1 µl Klenow Fragment, mix gently but thoroughly, and centrifuge briefly.

4. Incubate at 25°C for 1 h (see note 4).

5. Add 5 µl Stop Buffer.

6. Dilute a 2 µl-aliquot of the mixture with 498 µl of distilled water (provided by the user's laboratory) or with 498 µl of TE buffer [10 mM Tris-HCl, (pH 8), 1 mM Na_2 EDTA].

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

8. Dry the filter under a heat lamp, and determine the precipitable radioactivity by liquid scintillation counting. A result of X cpm in this test corresponds to a total of 2750 × cpm incorporated in the entire incubation mixture.

   For confirmation of total radioactivity in the incubation mixture, a second 5-µl aliquot of the above dilution (step 6) can be spotted on another glass fiber filter disk, dried under the heat lamp (without intervening TCA wash), and counted in a liquid scintillation counter. A result of X cpm in this test corresponds to 2750 × cpm of total radioactivity in the entire reaction mixture.

NOTES:

1. The Random Primers DNA Labeling System contains the deoxynucleoside triphosphates in four separate solutions. This allows the system to be used with any of the four [α-^{32}P]-labeled nucleoside triphosphates by suitably modifying the above protocol, which is given for the case of [α-^{32}P]-labeled 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 consistently used.

2. The Random Primers DNA Labeling System contains a qualified control DNA which may be used to monitor the performance of the system. The incorporation curve shown on the other side was obtained with this control DNA.

3. DNA probes labeled by this procedure can be used in blot hybridizations without removing the unincorporated nucleoside triphosphates (2). If desired, the labeled probe can be separated from unincorporated nucleotides by repeated ethanol precipitation (add 1/2 volume of 7.5 M ammonium acetate and 2 volumes of ethanol, repeat once.) or using the CONCERT™ PCR Purification System (Cat. Series 11458).

4. Incubation times longer than 1 h may give higher specific activity (see figure on other side).

5. For optimal results, supercoiled DNA should be linearized or alkali denatured prior to random promer labeling:

   -Effect of Substrate DNA Configuration/Denaturation on Labeling:

Substrate	Sp.Act.
Linear pUC 19 (denatured by boiling)	2.06×10^9 cpm/µg
Supercoiled pUC 19 (denatured by boiling)	0.12×10^9 cpm/µg
Supercoiled pUC 19 (alkali dematured))	1.30×10^9 cpm/µg

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.
3. Matathias, A.; and Komro, C. (1990) *FOCUS® 12:2*, 53.