Cat. # 6045

For Research Use

TaKaRa

Random Primer DNA Labeling Kit Ver.2.0

Product Manual

v201810Da

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I. Description

The Random Primer DNA Labeling Kit is designed to yield DNA probes with high specific activity for hybridization, and can be used to label DNA with [α - ^{32}P] or [^{3}H] dCTP. This kit is based on a modified method of Feinberg and Vogelstein[1),2)], utilizing 9 mer random oligonucleotide primers and the Exo - free Klenow fragment of *E.coli* DNA polymerase I which lacks 3' → 5' exonuclease activity. Each reaction is capable of generating DNA probes of high specific activity of 1 x 10^9 dpm/μg in as little as 2 - 3 minutes. It overcomes many of the disadvantages of the conventional nick translation procedure as shown in Table 1.

II. Components (30 reactions)

1.	Random Primer (9 mer)	60 μl
2.	10 x Buffer	75 μl
3.	dNTP Mixture (0.2 mM each dGTP, dATP, and dTTP)	75 μl
4.	Exo - free Klenow Fragment (2 U/μl)	30 μl
5.	Control DNA (λ - *Hin*d III Fragment) (25 ng/μl)	10 μl

【Materials Required but not Provided】

- Sterile purified water
- TE buffer (10 mM Tris - HCl, pH 8.0 and 1 mM EDTA)
- Labeled dCTP solution ; This kit is designed for use with [α - ^{32}P] dCTP (～ 111 TBq/mmol), however, [^{3}H] dCTP can also be used.

III. Storage

-20℃

V. Principles

DNA probes with high specific activity is used for detecting specific DNA sequences in many hybridization experiments.
The random primer labeling procedure reported by Feinberg and Vogelstein[1), 2)] produces probes with very high specific activity from small amounts of DNA (10 - 20 ng). As illustrated in Figure 1, the DNA to be labeled is heat denatured to produce single-stranded template. The random primers anneal to the single-stranded template DNA and are extended by the Klenow fragment as it incorporates labeled and unlabeled nucleotides.

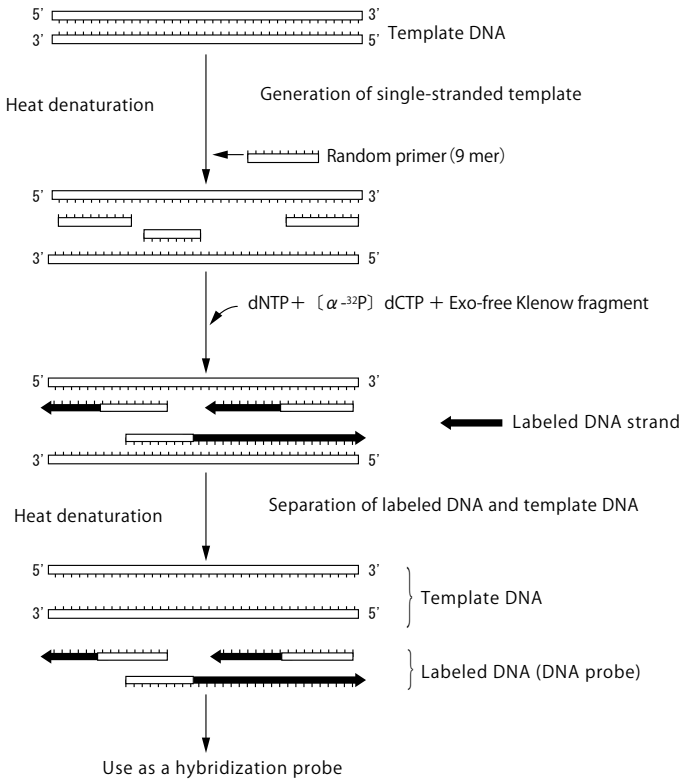

Figure 1. The principle of random primer labeling

Table 1

	Nick translation method	Random primer method
Incorporation rates	Decreases after prolonged incubation activity are obtained in a short time.	DNA probes with high specific Unaffected by prolonged incubation
Specific activity of product	$\sim 10^8$ dpm/μg	$\sim 10^9$ dpm/μg
Template impurities	Inhibited by the presence of agarose	Relatively unaffected by the presence of agarose
Purification after the reaction	Removal of unused dNTP is required	Removal of dNTP is not required
Amount of template required	≧ 1 μg	≧ 25 ng

These comparisons are based on the results of multiple control experiments using a λ - *Hin*d III Flagment that was labeled with 50 μCi of [α - ^{32}P] dCTP (370 MBq/ml)

V. Protocol

1) Add the following reagents in a microcentrifuge tube and bring the total volume to 14 μl with sterile purified water or TE buffer.

Reagents	Volume
Template DNA	10 ng - 1 μg ＊1
Random Primer	2 μl
Sterile purified water or TE buffer	x μl
Total	14 μl

2) Heat at 95℃ for 3 minutes and then cool on ice for 5 minutes.
3) Add 2.5 μl of 10 x Buffer, 2.5 μl of dNTP Mixture, and 5 μl of labeled dCTP.＊2 (1.85MBq , 50 μCi)
4) Add 1 μl of Exo - free Klenow Fragment and incubate at 37℃ for 10 minutes . ＊3
5) Inactivate the enzyme by heating the mixture at 65℃ for 5 minutes or adding EDTA in a final concentration of 30 mM.
6) Heat at 95℃ for 3 minutes and then cool on ice.
7) Use an appropriate volume as hybridization probe. (When necessary , unincorporated dCTP is removed by gel filtration or by ethanol precipitation.)

＊1 More than 300 bp of template DNA is suitable for the labeling with this kit. For labeling of DNA fragments shorter than 300 bp, it is recommended to use MEGALABEL™ (Cat. #6070), the kit for 5' - end labeling.
DNAs embedded in low melting temperature agarose gels can be used directly in the reaction without the removal of agarose. PrimeGel™ Agarose LMT 1-20K GAT (Cat. #5806A) or PrimeGel Agarose LMT PCR-Sieve GAT (Cat. #5815A) is suitable for this purpose. The procedure is as follows.

1) After agarose gel electrophoresis, excise a slice of the gel containing the target DNA fragment.
2) Add 3 volumes of sterile purified water to the agarose gel.
3) Melt the agarose at 65℃.
4) The solution corresponding to 25 ng of DNA can be used directly in the reaction as template DNA.

＊2 This kit is designed for use of 1.85 MBq (50 μCi) [α - ^{32}P] dCTP(～ 111 TBq/mmol, 370 MBq/ml), but [^{3}H] - labeled dCTP can also be used. When labeling with labeled dATP, use a mixture of dGTP, dCTP, and dTTP（0.2 mM each）instead of dNTP Mixture included in the kit.

＊3 A probe with sufficient specific radioactivity can be obtained after 2 - 3 minutes of incubation, but the highest specific radioactivity is obtained after 10 - 20 minutes incubation which also can be extended for an overnight without significant loss of activity. (Figure. 2)

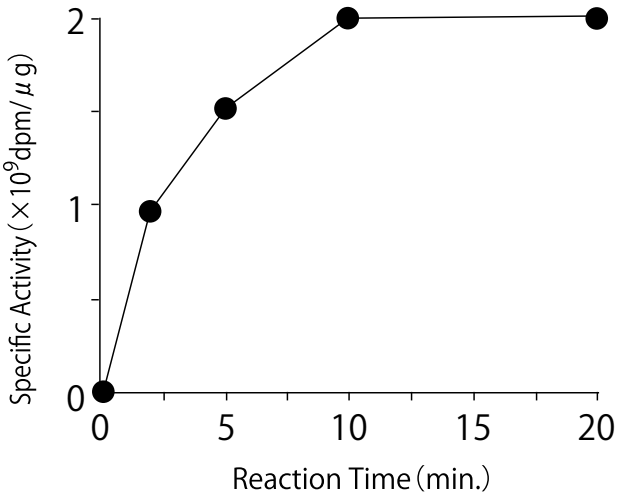

Figure 2.

(The result was obtained by using 25 ng of λ - *Hin*d III Fragment as the template)

VI. Effect of Amount of Template DNA

Amount of template DNA (ng)	10	25	100	1,000
Specific radioactivity of DNA (dpm / μg)	3.0 x 10^9	1.9 x 10^9	0.68 x 10^9	0.77 x 10^9

λ - *Hin*d III Fragment was labeled with 1.85 MBq (50 μCi) of [α - ^{32}P] dCTP (111 TBq/mmol, 370 MBq/ml), according to the Protocol.

VII. Measurement of Incorporation by DE81 - Filter Binding Assay

1) Dilute a small volume of the reaction mixture 20 - fold with TE buffer or sterile purified water.
2) Spot 3 μl of the diluted solution, in duplicate, onto DE81 paper discs (Whatman) and dry.
3) Wash one DE81 disc six times with 100 ml of 5% sodium phosphate, for 5 minutes each, twice with sterile purified water for 1 minute and twice with ethanol, and then dry. Measure the radioactivity with a liquid scintillation counter.
4) Measure the radioactivity of the other DE81 disc.
5) Incorporation yield and specific activity of DNA are calculated from the following formulas.

$$\text{Incorporation yield (\%)} = \frac{\text{(count of washed DE81 disc : cpm)}}{\text{(count of unwashed DE81 disc : cpm)}} \times 100$$

$$\text{Theoretical yield (ng)} = \frac{\mu\text{ Ci added x 4 x 330 ng/nmol}}{\text{Specific activity of labeled dNTP (}\mu\text{Ci / nmol)}}$$

If recommended amounts of labeled dCTP are used (50 μCi of [α - ^{32}P] dCTP with specific activity of 3,000 Ci/mmol), the theoretical yield of labeled probe will be 22 ng.

Total amount of probe (ng) = template DNA (ng) + incorporation yield x theoretical yield x 10^{-2}

$$\text{Specific radioactivity of probe (dpm/}\mu\text{g)} = \frac{2.2 \times 10^6 \times \mu\text{Ci added} \times \text{incorporation yield} \times 10^{-2}}{\text{total amount of probe (ng)} \times 10^{-3}}$$

VIII. References

1) Feinberg A P and Vogelstein B. *Anal. Biochem.* (1983) **132**: 6-13.
2) Feinberg A P and Vogelstein B. *Anal. Biochem.* (1984) **137**: 266-267.
3) Clark J M , Joyce C M, and Beardsley G P. *J Mol Biol.* (1987) **198**: 123-127.

IX. Related Products

[DNA labeling systems]
*Bca*BEST™ Labeling Kit (Cat #6046)
MEGALABEL™ (Cat #6070)