Cat. # 6046

For Research Use

TaKaRa

*Bca*BEST™ Labeling Kit

Product Manual

v201701Da

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I. Description

The *Bca*BEST Labeling Kit is a random primed labeling system[1), 2)] that uses longer primers and a thermostable DNA polymerase isolated from *Bacillus caldotenax* (*Bca*BEST DNA polymerase). Unlike the *E. coli* Klenow fragment or modified T7 DNA polymerase, *Bca*BEST DNA polymerase allows stable polymerization through templates with high-GC content or with higher order structure. The longer 9 mer primers increase the efficiency of incorporation of the labeled nucleotides up to 80% (Table 1). With this kit, random priming is accomplished in 10 minutes to create DNA probes that are efficiently labeled. The kit is designed for use with [α-^{32}P] or [^{3}H] dCTP labels. Generally, a probe with specific activity of >10^9 dpm/ μg DNA will be obtained with [α-^{32}P] dCTP (～ 370 MBq/ml : 111 TBq/mmol).

Table 1

	Random Primer DNA Labeling Kit Ver.2	*Bca*BEST Labeling Kit
Reaction time	10 minutes	10 minutes
Specific activity of product	～ 10^9 dpm/ μg	～ 10^9 dpm/ μg
Extension capacity	Decreases with GC-rich templates	On effect with GC-rich templates

This comparison was made by using a λ DNA-*Hin*d III template, that was labeled with 50 μCi of [α-^{32}P] dCTP (370 MBq/ml). If the template is ≦ 300 bp, incorporation rate may decrease for both kits.

II. Components (For 40 reacsions)

1. Random primer (9 mer) 80 μl
2. 10X Buffer 100 μl
3. dNTP mixture (dATP, dGTP, and dTTP) (each 0.2 mM) 100 μl
4. *Bca*BEST DNA polymerase (2 U/ μl) 40 μl
   (cloned and modified *B. caldotenax* DNA polymerase)
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, 1 mM EDTA)
- Labeled dCTP

  This kit is designed for use with [α-^{32}P] dCTP (111 TBq/mmol, 370 MBq/ml)*, however, [^{3}H] dCTP can also be used. When using [^{3}H] dCTP, adjust the concentration of labeled dCTP to 16.5 pmol.

  ＊ The current name is dCTP, [α-^{32}P]- 3000 Ci/mmol 10 mCi/ml.

III. Storage -20℃

IV. Principles

DNA probes with high specific activity are required for detection of specific DNA sequences in many hybridization experiments. Random primer labeling reported by Feinberg and Vogelstein[1), 2)] produces probes with very high specificity from small amounts of DNA (10 - 20 ng) and can be used to label DNA fragments even directly from low melting temperature agarose gel slices. As illustrated in the schematic below, the DNA to be labeled is heat denatured to produce a single-stranded template. The random primers anneal to the single-stranded template DNA and are extended by *Bca*BEST DNA polymerase to incorporate labeled and unlabeled nucleotides.

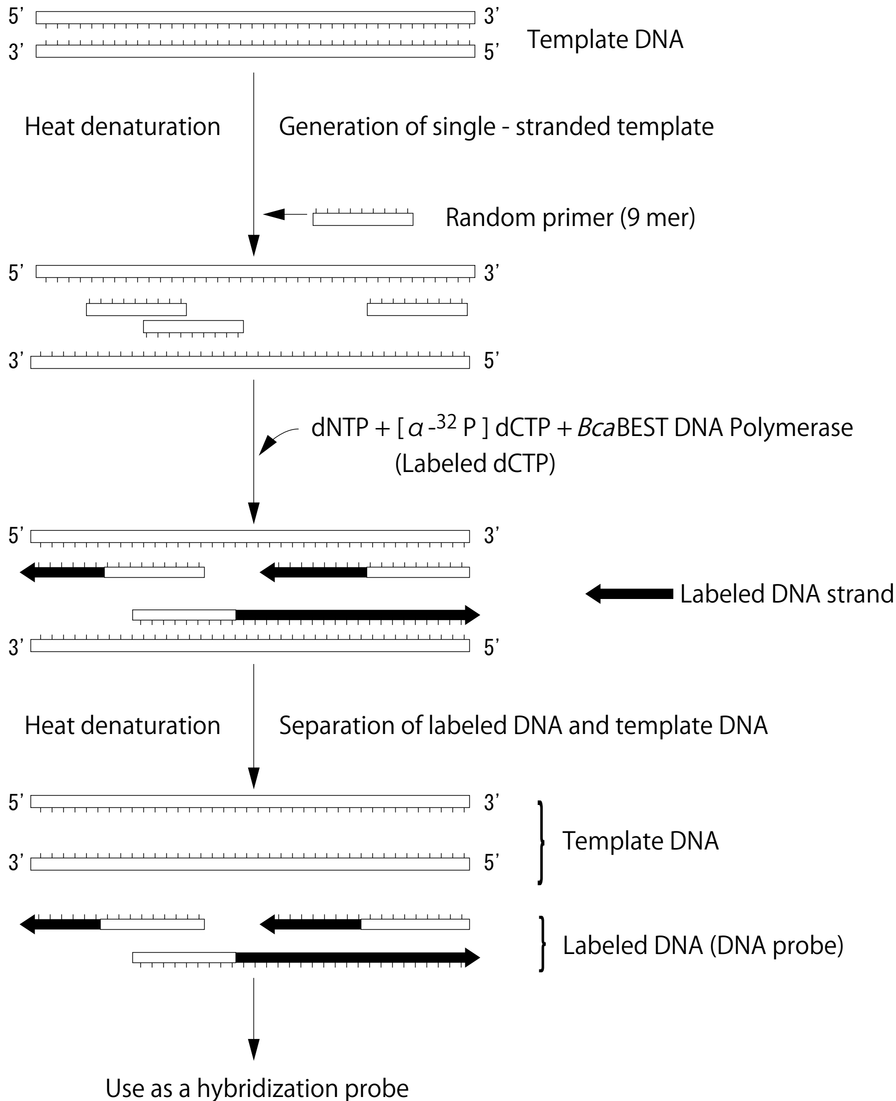

Figure 1. Principle of random primed labeling

V. Protocol

1) Combine the following components in a microcentrifuge tube.

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

2) Heat at 95℃ for 3 minutes and then cool on ice for 5 minutes.

3) Add 2.5 μl of 10X buffer, 2.5 μl of the dNTP mixture and 5 μl of labeled dCTP*2 (1.85 MBq, 50 μCi)

4) Add 1 μl of *Bca*BEST DNA Polymerase and incubate at 50 - 55℃ for 10 minutes.*3

5) Inactivate the enzyme by adding EDTA to a final concentration of 30 mM.

6) Heat at 95℃ for 3 minutes and then cool on ice.

7) Use an appropriate volume as a hybridization probe. (When necessary, unincorporated dCTP can be removed by gel filtration, centrifugal filter or by ethanol precipitation.)

*1 Template DNA
This kit is designed to use 10 ng - 1 μg of template DNA. DNA in low melting temperature agarose gels can be used directly in the reaction without removal of agarose. 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 slice.
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 Labeled dCTP
This kit is designed for use of 1.85 MBq (50 μCi). [α-^{32}P] dCTP (~111 TBq/mmol, 370 MBq/ml) and [^{3}H] - labeled dCTP can also be used.
In this case adjust dCTP amount 16.5 pmol. When labeling with labeled dATP, use a solution of dGTP, dCTP, and dTTP (0.2 mM each) instead of the dNTP mixture included in the kit.

*3 Incubation time
A probe with sufficient specific radioactivity can be obtained after 10 minutes of incubation. Incubation time also can be extended for an overnight without significant loss of activity. If specific activity is low, it is possible to for an overnight.

VI. Effect of Template DNA Amount

Amount of template DNA (ng)	10	25	100	1,000
Specific radioactivity of probe (dpm/ μ g)	2.9×10^9	1.8×10^9	0.68×10^9	0.78×10^8

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

VII. Measurement of Incorporation

1) Dilute a small volume of the reaction mixture 20 - fold with TE buffer or sterile purified water.
2) Spot 3 μ l of the diluted mixture, in duplicate, onto DE81 paper discs (GE Healthcare) and dry.
3) Wash one DE81 disc in 100 ml of 0.5 M sodium phosphate, pH 6.8 six times for 5 minutes each, twice with sterile purified water for 1 minute, and twice with ethanol, then dry. Measure the radioactivity with a liquid scintillation counter.
4) Measure the radioactivity of the other DE81 disc.
5) The incorporation yield and the specific activity of probe are calculated from the following formulas.

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

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

If recommended amounts of labeled dCTP are used (50 μ Ci of [α -^{32} P] dCTP with specific activity of 370 MBq/ml), the theoretical yield of labeled probe will be 22 ng.

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

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

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) Uemori, T., Ishino, Y., Fujita, K., Asada, K., and Kato, I. *FASEB J.* (1992) **6**: A216.

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