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Roche

High Prime DNA Labeling Kit

For radioactive and nonradioactive labeling of DNA with any modified deoxyribonucleoside-triphosphate using random oligonucleotides as primers

Cat. No. 11 585 584 001

Kit for 50 labeling assays

Version 10
Content version: July 2016

Store at −15 to −25°C

1. What this Product Does

Number of Tests

1 kit is sufficient for 50 labeling assays using 0.01 -2 µg DNA.

Kit Contents

Bottle	Label	Content/ Function
1	High Prime reaction mixture 5× conc.	• 200 µl random primer mixture • [1 U/µl Klenow polymerase, labeling grade, and 5× stabilized reaction buffer in 50% (v/v) glycerol].
2	dATP	50 µl 0.5 mM 2'-deoxyadenosine-5'-triphosphate in Tris buffer.
3	dCTP	50 µl 0.5 mM 2'-deoxycytidine-5'-triphosphate in Tris buffer.
4	dGTP	50 µl 0.5 mM 2'-deoxyguanosine-5'-triphosphate in Tris buffer.
5	dTTP	50 µl 0.5 mM thymidine-5'-triphosphate in Tris buffer.
6	Control DNA	• 20 µl λDNA • [12.5 µg/ml]

Application

The method enables the labeling of DNAs available only in minute quantities, *e.g.,* DNA restriction fragments isolated from gels or in molten agarose.

High Prime labeled probes are used in a variety of hybridization techniques:

- Southern blots (3)
- Northern blots (4)
- screening of gene libraries (5)
- *in situ* hybridizations.

Stability and Storage

The unopened vial is stable at –15 to -25°C until the expiration date printed on the label.

⚠ Repeated freezing and thawing should be avoided. To avoid contamination we recommend to aliquot the High Prime reaction mixture (vial 1) and store in 2-3 portions.

2. How to Use this Product

2.1 Before you Begin

When varying the ratio of substrate DNA to labeled deoxyribonucleoside-triphosphate, similar incorporation rates, but different levels of specific activity are obtained (see sections 2 and 3).

Sample Material

The input DNA can be:

- either linear or supercoiled plasmid DNA, λDNA
- shorter fragments of 200 bp
- DNA fragments in molten agarose
- < 10 ng DNA

⚠ The length of the DNA to be labeled does not influence the reaction. Maximal incorporation may be achieved only after longer incubation of 30-60 min.

Preparation of Working Solution

dATP, dGTP, dTTP mixture:

For one labeling reaction pipette:

1 µl dATP, (vial 2)

1 µl dGTP, (vial 4)

1 µl dTTP, (vial 5)

to a reaction vial.

⚠ If the same type of labeled deoxyribonucleoside-triphosphate is used repeatedly, we recommend the preparation of a mixture of equal parts of the other three deoxyribonucleoside-triphosphates for convenience.

Removal of Non-incorporated Radioactivity

Removal of non-incorporated deoxyribonucleoside-triphosphates prior to hybridization is not necessary, but can be performed by chromatography on Quick Spin Columns, Sephadex G-50 Fine* or by ethanol precipitation, if desired.

2.2 Standard Labeling Assay

Procedure

In the following table please find a protocol for the standard labeling assay.

❶ Add 25 ng template DNA (linear or supercoiled) and sterile, double dist. water to a final volume of 11 µl to a microfuge tube. For the control reaction use 2 µl control DNA (vial 6) and 9 µl PCR grade water.

❷ Denature the DNA by heating in a boiling water bath for 10 min and chilling quickly in an ice/water bath.

⚠ Complete denaturation is essential for efficient labeling.

❸ • Centrifuge briefly the denatured DNA and add the following components on ice:

Reagent	Vol.
High Prime reaction mixture (vial 1)	4 µl
dATP, dGTP, dTTP mixture	3 µl
20 µCi [α^{32}P]dCTP, 3000 Ci/mmol, aqueous solution	2 µl

• Mix and centrifuge briefly.

❹ Incubate for 10 min at 37°C.

❺ Stop the reaction by adding 2 µl 0.2 M EDTA (pH 8.0) and/or by heating to 65°C for 10 min.

2.3 Labeling Assay using Low-Melting Point Agarose

Procedure

Please refer to the following table.

❶ Excise the DNA fragment to be labeled cleanly from a low-melting point agarose gel and transfer it to a 1.5 ml microfuge tube.

❷ • Add PCR grade water to a ratio of 3 ml/g gel and heat the tube for 7 min at 100°C to melt the gel and denature the DNA.
• After cooling to 37°C the DNA/agarose mixture can be used directly for labeling.

❸ • Add to the denatured DNA on ice:

Reagent	Vol.
High Prime reaction mixture (vial 1)	4 µl
dATP, dGTP, dTTP mixture	3 µl
20 µCi [α^{32}P]dCTP, 3000 Ci/mmol, aqueous solution	2 µl

• Mix and centrifuge briefly.

❹ Incubate for 60 min at 37°C.

❺ Stop the reaction by adding 2 µl 0.2 M EDTA (pH 8.0) and/or by heating to 65°C for 10 min.

2.4 Labeling Assay using DIG-dUTP

Other nonradioactive labels (*e.g.,* biotin, fluorescein) can be incorporated replacing DIG-dUTP by the appropriate modified deoxyribonucleoside-triphos-phate in the labeling reaction described below.

Also for the nonradioactive labeling assays the non-incorporated nucleotides do not have to be removed prior to hybridization.

Procedure

Please refer to the following table.

❶ Add 10 ng – 3 µg template DNA (linear or supercoiled) and PCR grade water to a final volume of 12 µl to a microfuge tube.

❷ Denature the DNA by heating in a boiling water bath for 10 min and chilling quickly on ice.

⚠ Complete denaturation is essential for efficient labeling. Depending on the DNA used a much shorter denaturing time down to 1 min has proved to be efficient, e.g., for λDNA we recommend 1 to 2 min at 95°C.

❸ • Centrifuge briefly the denatured DNA and add the following components on ice:

Reagent	Vol.
High Prime reaction mixture (vial 1)	4 µl
DIG DNA Labeling Mix, containing 1 mM dATP, dCTP, dGTP, 0.65 mM dTTP, 0.35 mM DIG-dUTP.	4 µl

• Mix and centrifuge briefly.

❹ Incubate for 60 min at 37°C.

⚠ Longer incubation (up to 20 h) can increase the yield of labeled DNA.

❺ Stop the reaction by adding 2 µl 0.2 M EDTA (pH 8.0) and/or by heating to 65°C for 10 min.

Labeling Efficiency

The yield of DIG-labeled DNA from 1 µg template DNA is approx. 0.8 µg labeled DNA after 1 h and 2 µg after 20 h. The yields for biotin- and fluorescein-labeling are slightly lower.

3. Results

3.1 Data analysis

Determination of labeling degree

The degree of labeling is determined by comparison of incorporated to total input radioactivity in an aliquot of the reaction. The kinetics of the reaction may be followed by precipitation of the DNA with trichloroacetic acid of aliquots removed at various time points during the reaction.

Newly synthesized DNA

The amount of newly synthesized DNA (ng) is determined as follows:

$$\frac{\mu\text{Ci dNTP} \times 13.2 \times \%\text{ incorporation}}{\text{specific activity of dNTP (Ci/mmol)}}$$

Amount of incorporated radioactivity

The amount of incorporated radioactivity in dpm is:

$$\mu\text{Ci dNTP} \times 2.2 \times 10^4 \times \%\text{ incorporation.}$$

⚠ In the standard assay 75% incorporation is obtained either with [α^{32}P]dCTP, 3000 Ci/mmol or with [α^{32}P]dCTP, 6000 Ci/mmol.

Amount of specific activity

The specific activity in dpm/µg is calculated according to the following formula:

$$\frac{\text{incorporated radioactivity} \times 10^3}{(\text{input DNA} + \text{newly synthesized DNA [ng]})}$$

3.2 Typical results

Typical experiment

Using the High Prime Labeling Kit labeling reactions were performed as follows:

25 and 100 ng λDNA were labeled with 20, 50 and 100 μCi [α^{32}P]dCTP, 3000 Ci/mmol;

1000 ng λDNA were labeled with 50 μCi [α^{32}P]dCTP, 3000 Ci/mmol;

25 ng λDNA were labeled with 50 and l00 μCi [α^{32}P]dCTP, 6000 Ci/mmol.

The incorporation rates and specific activities obtained are given below

λDNA	20 μCi	50 μCi	100 μCi	[α^{32}P]dCTP, 3000 Ci/mmol
25 ng	77% 1.1×10^9	75% 2×10^9	72% 2.8×10^9	incorporation dpm/μg
100 ng	79% 3.2×10^8	78% 7.3×10^8	77% 1.3×10^9	incorporation dpm/μg
1000 ng		63% 6.8×10^7		incorporation dpm/μg

λDNA	50 μCi	100 μCi	[α^{32}P]dCTP, 6000 Ci/mmol
25 ng	75% 2.5×10^9	72% 3.9×10^9	incorporation dpm/μg

The reaction kinetics with the new High Prime reaction mixture are very fast (see fig. 1 and 2). Similar results are obtained using 25 and 100 ng DNA. The lengths of the radioactive fragments obtained by High Prime reactions are on the average 80-120 bp, irrespective of the size of the input DNA.

Figure 1

Kinetics of incorporation 25 ng λDNA were labeled with 20 μCi [α^{32}P]dCTP, 3000 Ci/mmol.

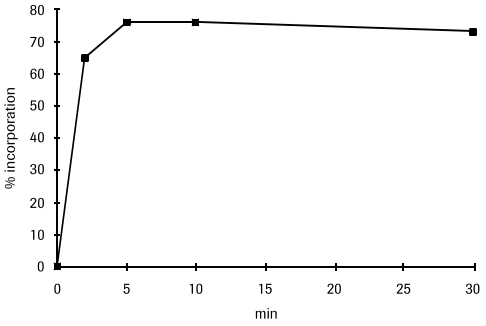
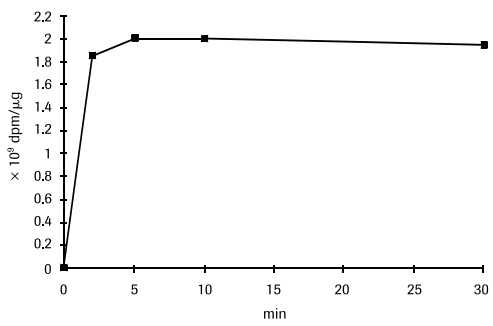


Figure 2

Kinetics of specific activity. 25 ng λDNA were labeled with 20 μCi [α^{32}P]dCTP, 3000 Ci/mmol.



4. Additional Information on this Product

4.1 How this Product Works

The method of "random primed" DNA labeling originally developed by Feinberg and Vogelstein (1, 2) is based on the hybridization of oligonucleotides of all possible sequences to the denatured DNA to be labeled.

The input DNA serves solely as template for the synthesis of labeled DNA, and is not degraded during the reaction, making it possible to label minimal amounts of DNA (10 ng) with this method. Practically all sequence combinations are represented in the oligonucleotide random primer mixture, so the primers bind to the template in a statistical manner. Thus, an equal degree of labeling along the entire length of the input DNA is guaranteed. Random primed labeling with the new High Prime is both faster and more efficient than with the traditional random primed labeling.

4.2 Product Characteristics

Labeling Principle

The complementary DNA strand is synthesized by Klenow polymerase using the 3'OH termini of the random oligonucleotides as primers. Modified deoxyribonucleoside-triphosphates (*e.g.,* labeled with ^{32}P, ^{35}S, ^{3}H, digoxigenin, biotin, fluorescein, or rhodamin) present in the reaction are incorporated into the newly synthesized complementary DNA strand.

Composition of the Labeling Mixture

High Prime Labeling Kit uses a specially developed reaction mixture containing random oligonucleotides, Klenow polymerase, labeling grade and an optimized reaction buffer concentrate in 50% glycerol for rapid and efficient labeling of DNA.

This kit is specially designed for DNA labeling with any type of radioactive or nonradioactive labeled deoxyribonucleoside-triphosphate. For labeling using radioactive dCTP we provide a highly convenient readily mixed High Prime*.

Incorporation

In the standard assay 75% incorporation is obtained either with [α^{32}P]dCTP, 3000 Ci/mmol or with [α^{32}P]dCTP, 6000 Ci/mmol.

Specific Activity

The standard assay will routinely give a specific activity of 2×10^9 dpm/μg, with different substrate DNAs in 10 min.

4.3 Quality Control

In the standard assay with 25 ng pBR322 Plasmid DNA and 20 μCi [α^{32}P]dCTP, 3000 Ci/mmol, an incorporation rate of >60% = 1.8×10^9 dpm/μg is obtained after 10 min incubation at 37°C.

4.4 References

1 Feinberg, A.P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6.
2 Feinberg, A.P. & Vogelstein, B. (1984) *Anal. Biochem.* **137,** 266.
3 Southern, E.M. (1975) *J. Mol. Biol.* **98,** 503.
4 Smith, G.E. & Summers, M.D. (1980) *Anal. Biochem.* **109,** 123.
5 Grunstein, M. & Hogness, D. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3961.

5. Supplementary Information

5.1 Conventions

Text Conventions
To make information consistent and memorable, the following text conventions are used in this package insert:

Text Convention	Use
Numbered Instructions labeled ①, ②,etc.	Steps in a process that usually occur in the order listed
Numbered Instructions labeled ❶, ❷,etc.	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Diagnostics

Symbols
In this package insert the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

5.2 Ordering Information

Kits

Product	Pack size	Cat. No.
High Pure PCR Product Purification Kit	1 kit for 50 purifications	11 732 668 001
Agarose Gel DNA Extraction Kit	1 kit (max. 100 reactions)	11 696 505 001
DIG DNA Labeling Kit	1 kit for 40 reactions	11 175 033 910
DIG-High Prime	160 µl (40 reactions)	11 585 606 910
DIG Luminescent Detection Kit	1 kit for 50 blots	11 363 514 910

Single reagents

Product	Pack size	Cat. No.
Klenow Enzyme	100 U 500 U	11 008 404 001 11 008 412 001
High Prime	200 µl	11 585 592 001
DIG-High Prime	160 µl	11 585 606 910
Fluorescein-High Prime	100 µl	11 585 622 910
Biotin-High Prime	100 µl	11 585 649 910
Quick Spin Columns, Sephadex G-50 (Fine)	20 columns 50 columns	11 273 965 001 11 273 973 001

5.3 Changes to previous version

- Editorial changes
- see 2.2 Standard Labeling Assay: Procedure.

5.4 Trademarks

Disclaimer of License

For patent license limitations for individual products please refer to: List of biochemical reagent products

Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

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