High Prime

For the labeling of DNA with radioactive dCTP using random oligonucleotides as primers

Cat. No. 11 585 592 001 200 µl

Premixed solution for 50 labeling assays

Version 11

Content version: January 2019

Store at −15 to −25°C

1. What this Product Does

Contents

Label	Content
High Prime	• 200 µl • 5× conc. random primer mix: 1 U/µl Klenow polymerase, labeling grade, 0.125 mM dATP, 0.125 mM dGTP, 0.125 mM dTTP in 50% (v/v) glycerol

Labeling Principle

Labeled probes are generated with High Prime according to the random primed labeling technique (1,2). High Prime is a specially developed reaction mixture containing random oligonucleotides, Klenow polymerase, labeling grade, dATP, dGTP and dTTP in an optimized reaction buffer concentrate in 50% glycerol for rapid and efficient labeling of DNA with ^{32}P-, ^{35}S-, or ^{3}H-labeled dCTP. The premixed High Prime solution reduces pipetting steps and increases yield, reproducibility and convenience.

Application

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

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

Sample Material

- DNA fragments of at least 100 bp
- linearized plasmid, cosmid or λDNA
- supercoiled DNA
- or minimal amounts of DNA (10 ng), *e.g.* DNA restriction fragments isolated from gels or in molten agarose

The length of the DNA to be labeled does not influence the reaction. Maximal incorporation may require a prolonged incubation period of 30-60 min.

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.

Quality Control

In the standard assay with 25 ng λDNA 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.

Stability

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

Avoid repeated freezing and thawing. To avoid contamination we recommend to aliquot the High Prime solution and to store in 2 – 3 portions.

2. How to Use this Product

Standard Labeling Assay
Additional Equipment and Reagents Required

- water bath
- ice/water
- 2 µl 20 µCi [α^{32}P]dCTP, 3000 Ci/mmol, aqueous solution
- 0.2 M EDTA (pH 8.0)

Procedure

A protocol for the standard labeling assay is provided in the following table.

Step	Action
❶	Add 25 ng **template DNA** (linear or supercoiled) and sterile, double dist. water to a final volume of 14 µl to a reaction vial.
❷	Denature the DNA by heating in a boiling water bath for 10 min and chilling quickly in an ice/water bath. ***Note***: 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	• Mix **High Prime** thoroughly and add 4 µl to the denatured DNA. • Add 2 µl 20 µCi **[α^{32}P]dCTP**, • Mix and centrifuge briefly. • Incubate for 10 min at +37°C.
❹	Stop the reaction by adding 2 µl **0.2 M EDTA** (pH 8.0) and/or by heating to +65°C for 10 min.

Removal of non-incorporated Radioactivity

For the removal of unincorporated deoxyribonucleoside triphosphates we recommend the use of:

- Quick Spin Column, Sephadex G-50 (Fine) or
- repeated ethanol precipitation.

Labeling of DNA in Low-Melting Point Agarose
Procedure

The procedure for the labeling of DNA isolated from low-melting point agarose is described in the following table.

Step	Action
❶	Neatly excise the **DNA fragment** to be labeled from a low-melting point agarose gel and transfer it to a 1.5 ml reaction vial.
❷	• Add **sterile, double dist. 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	• Mix **High Prime** thoroughly and add 4 µl to the denatured DNA. • Add 5 µl 20 µCi **[α^{32}P]dCTP**, • Mix and centrifuge briefly. • Incubate for 60 min at +37°C.
❹	Stop the reaction by adding 2 µl **0.2 M EDTA** (pH 8.0) and/or by heating to +65°C for 10 min.

3. Results

Data Analysis
Determination of the Labeling Degree

The degree of labeling is determined by comparing the incorporated radioactivity to the 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.

Calculation of labeling parameters

Please refer to the following table.

Calculation of...	Formula
newly synthesized DNA (ng)	$\frac{\mu Ci\ dNTP \times 13.2 \times \%\ incorporation}{specific\ activity\ of\ dNTP\ (Ci/mmol)}$
incorporated activity (dpm)	$\mu Ci\ dNTP \times 2.2 \times 10^4 \times \%$ incorporation
specific activity (dpm/µg)	$\frac{incorporated\ radioactivity \times 10^3}{(input\ DNA + newly\ synthesized\ DNA\ [ng])}$

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 100 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%	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 average lengths of the radioactive fragments yielded by High Prime reactions are 80–120 bp, irrespective of the size of the input DNA.

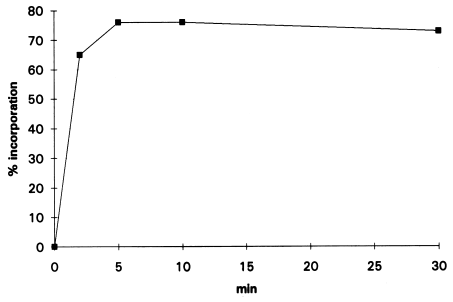

Figure 1: Kinetics of incorporation. 25 ng λDNA were labeled with 20 µCi [α^{32}P] dCTP, 3000 Ci/mmol.

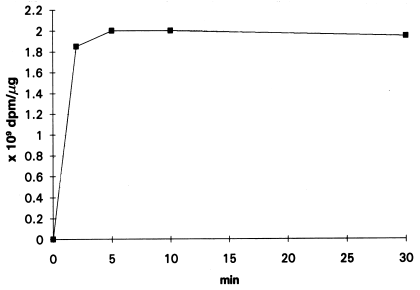

Figure 2: Kinetics of specific activity. 25 ng λDNA were labeled with 20 µCi [a^{32}P]dCTP, 3000 Ci/mmol.

Changes to previous version

- Editorial changes.

References

1 Feinberg, A.P. & Vogelstein, B.(1983) *Anal. Biochem.* **132**, 6.
2 Feinberg, A.P. & Vogelstein, B.(1984) *Anal. Biorhem.* **137**, 266.
3 Southern, E.M. (1975) *J. Mol. Biol.* **98**, 503.
4 Smith, G.E. & Summers, M.D. (1990) *Anal. Biochem.* **109**,123.
5 Grunstein, M. & Hogness, D. (1975) *Proc. Natl. Acad. Sci.* USA **72**, 3961.

Ordering Information

Kits

Product	Pack Size	Cat. No.
Random Primed DNA Labeling Kit	1 kit (for 50 labeling reactions)	11 004 760 001
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
High Prime DNA Labeling Kit	1 kit for 50 reactions	11 585 584 910
DIG DNA Labeling Kit	1 kit for 40 reactions	11 175 033 910
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
DIG-High Prime	160 µl (for 40 labeling reactions)	11 585 606 910
Fluorescein-High Prime	100 µl (25 reactions)	11 585 622 910
Biotin-High Prime	100 µl (4× 100 µl)	11 585 649 910
Quick Spin Columns for radio-labeled DNA purification, Sephadex G-50	20 columns	11 273 965 001
Digoxigenin-11-dUTP, alkali stable	25 nmol (25 µl) 125 nmol (125 µl)	11 093 088 910 11 558 706 910
Digoxigenin-11-dUTP, alkali labile	25 nmol (25 µl) 125 nmol (125 µl)	11 573 152 910 11 573 179 910
Biotin-16-dUTP	50 nmol	11 093 070 910
Fluorescein-12-dUTP	25 nmol	11 373 242 910
Tetramethyl-rhodamine-6-dUTP	25 nmol	11 534 378 910
DIG DNA Labeling Mix, 10x	50 µl (25 reactions)	11 277 065 910
dATP, lithium salt	25 µmol (250 µl)	11 051 440 001
dCTP, lithium salt	25 µmol (250 µl)	11 051 458 001
dGTP, lithium salt	25 µmol (250 µl)	11 051 466 001
dTTP, lithium salt	25 µmol (250 µl)	11 051 482 001
Desoxynucleoside Triphosphate Set	4x 10 µmol (100 µl)	11 277 049 001

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