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

High Prime

Version: 12
Content Version: November 2020

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

Cat. No. 11 585 592 001 200 µl
50 labeling assays

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	High Prime, 5x conc.	Random prime labeling mixture: Premixed solution of 1 U/µl Klenow polymerase, labeling grade, 0.125 mM dATP, dGTP, dTTP each in 50% (v/v) glycerol.	1 vial, 200 µl

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	High Prime, 5x conc.	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.*** ⚠ ***To avoid contamination, aliquot and store the solution in 2 to 3 vials.***

1.3. Additional Equipment and Reagent required

For random primed DNA labeling

- Water bath
- Ice bath
- Autoclaved, double-distilled water
- 2 µl 20 µCi α^{32}P-dCTP, 3,000 Ci/mmol, aqueous solution
- 0.2 M EDTA, pH 8.0

For removal of unincorporated radioactivity

- Quick Spin Columns for radiolabeled DNA purification Sephadex G-50*

1.4. Application

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

- Southern blots
- Northern blots
- Dot/slot blots
- Screening of gene libraries
- *In situ* hybridizations

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Templates for labeling reaction

- DNA fragments of at least 100 bp.
- Linearized plasmid, cosmid, or λDNA.
- Supercoiled DNA
- Minimal amounts of DNA (10 ng), such as 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 to 60 minutes.***

Safety Information

For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

Working Solution

Solution	Composition	Use
Water	Autoclaved, double-distilled water	Dilution of DNA.
EDTA	0.2 M ethylenediaminetetraacetic acid, pH 8.0	Stops the reaction.

2.2. Protocols

Random primed DNA labeling

Perform the standard random primed DNA labeling according to the following steps.

1. To a microfuge tube, add 25 ng template DNA (linear or supercoiled) and autoclaved, double-distilled water to a final volume of 14 µl.

2. Denature the DNA by heating in a boiling water bath for 10 minutes.
   – Quickly chill in an ice water bath.
   i *Full denaturation is essential for efficient labeling. Depending on the DNA used, a much shorter denaturing time down to 1 minute has proved to be efficient, for example, for λDNA, use 1 to 2 minutes at +95°C.*

3. 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 minutes at +37°C.

4. Stop the reaction by adding 2 µl 0.2 M EDTA, pH 8.0, and/or by heating to +65°C for 10 minutes.

Removal of non-incorporated radioactivity

Remove unincorporated deoxyribonucleoside-triphosphates using:
- Quick Spin Columns for radiolabeled DNA purification Sephadex G-50*, or
- Repeated ethanol precipitation.

Labeling DNA in low-melting point agarose

1. Neatly excise the DNA fragment to be labeled from a low-melting point agarose gel and transfer it to a 1.5 ml microfuge tube.

2. Add autoclaved, double-distilled water to a ratio of 3 ml/g gel, and heat the tube for 7 minutes 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.

3. 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 minutes at +37°C.

4. Stop the reaction by adding 2 µl 0.2 M EDTA, pH 8.0, and/or by heating to +65°C for 10 minutes.

2.3. Parameters

Inactivation

+65°C
Stop the reaction by adding 2 µl 0.2 M EDTA, pH 8.0, and/or by heating to +65°C for 10 minutes.

Specific Activity

The standard assay routinely yields a specific activity of 2 x 10^9 dpm/µg, using different substrate DNAs after 10 minutes of incubation.

3. Results

Incorporation

In the standard assay, 75% incorporation is obtained with either α^{32}P-dCTP, 3,000 Ci/mmol or with α^{32}P-dCTP, 6,000 Ci/mmol.

Determination of labeling degree

The degree of labeling is determined by comparing the 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.

Calculation of the labeling parameters

Newly synthesized DNA

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

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

Amount of incorporated radioactivity

The amount of incorporated radioactivity in dpm is:

$$\mu Ci\ dNTP \times 2.2 \times 10^4 \times \%\ incorporation$$

Amount of specific activity

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

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

Typical experiment

Using the High Prime, labeling reactions were performed as shown below:
- 25 and 100 ng λDNA was labeled with 20, 50, and 100 µCi α^{32}P-dCTP, 3,000 Ci/mmol.
- 1,000 ng λDNA was labeled with 50 µCi α^{32}P-dCTP, 3,000 Ci/mmol.
- 25 ng λDNA was labeled with 50 and 100 µCi α^{32}P-dCTP, 6,000 Ci/mmol.

Incorporation rates and specific activities

λDNA [ng]	20 µCi	50 µCi	100 µCi	α^{32}P-dCTP, 3,000 Ci/mmol
25	77% 1.1 × 10^9	75% 2 × 10^9	72% 2.8 × 10^9	incorporation dpm/µg
100	79%	78% 7.3 × 10^8	77% 1.3 × 10^9	
1,000	–	63% 6.8 × 10^7	–	
λDNA [ng]	**20 µCi**	**50 µCi**	**100 µCi**	**α^{32}P-dCTP, 6,000 Ci/mmol**
25	–	75% 2.5 × 10^9	72% 3.9 × 10^9	incorporation dpm/µg

The reaction kinetics with the High Prime reaction mixture are very fast, see Figures 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 to 120 bp, irrespective of the size of the input DNA.

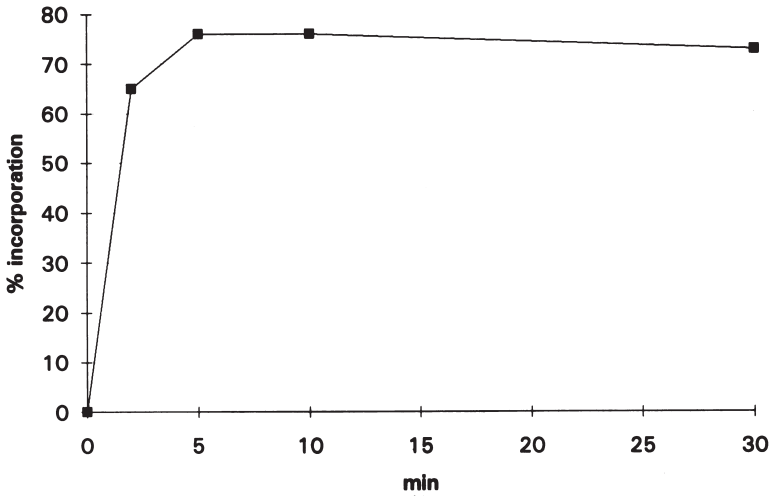

Fig. 1: Kinetics of incorporation. 25 ng λDNA was labeled with 20 µCi α^{32}P-dCTP, 3,000 Ci/mmol.

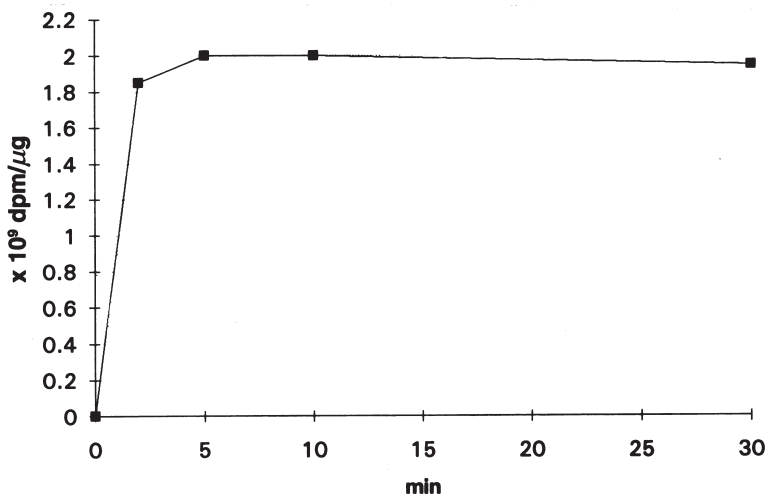

Fig. 2: Kinetics of specific activity. 25 ng λDNA was labeled with 20 µCi α^{32}P-dCTP, 3,000 Ci/mmol.

4. Additional Information on this Product

4.1. Test Principle

How this product works

The random primed DNA labeling method is based on the hybridization of oligonucleotides of all possible sequences to the denatured DNA to be labeled.

- Input DNA is the only template for synthesis of labeled DNA and is not degraded during the reaction, making it possible to label minimal amounts of DNA (10 ng) using this method.
- Practically all sequence combinations are represented in the oligonucleotide random primer mixture, therefore 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.

Labeling principle

① Complementary DNA strands are synthesized using Klenow polymerase at the 3′-OH termini of randomized oligonucleotides used as primers.

② Modified deoxyribonucleoside-triphosphates, such as labeled with ^{32}P, ^{35}S, ^{3}H, digoxigenin, biotin, fluorescein, or rhodamine, added to the reaction are readily incorporated into newly synthesized DNA strands.

4.2. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.
New information added related to the REACH Annex XIV.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Quick Spin Columns for radiolabeled DNA purification	20 columns	11 273 965 001
	50 columns	11 273 973 001

5.4. Trademarks

All product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

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

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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