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Random Primed DNA Labeling Kit

Version: 24

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For labeling DNA using random oligonucleotides as primers.

Cat. No. 11 004 760 001 1 kit
50 labeling assays

Store the kit at −15 to −25°C.

1. General Information 3
1.1. Contents 3
1.2. Storage and Stability 3
Storage Conditions (Product) 3
1.3. Additional Equipment and Reagent required 4
For DNA purification from agarose gels 4
For removal of non-incorporated radioactivity 4
1.4. Application 4
1.5. Preparation Time 4
Assay Time 4

2. How to Use this Product 5
2.1. Before you Begin 5
Sample Materials 5
Templates for labeling reaction 5
General Considerations 5
Labeling efficiency 5
Labeling variations 5
Working Solution 5
2.2. Protocols 6
Labeling DNA using α^{32}P-dCTP 6
Labeling DNA using Digoxigenin-11-dUTP* 6
Removal of non-incorporated nucleotides 7
2.3. Parameters 7
Specific Activity 7

3. Results 8
Data analysis 8
Typical results 8

4. Troubleshooting 10

5. Additional Information on this Product 11
5.1. Test Principle 11
How this product works 11
Labeling principle 11
5.2. Quality Control 11

6. Supplementary Information 12
6.1. Conventions 12
6.2. Changes to previous version 12
6.3. Ordering Information 12
6.4. Trademarks 13
6.5. License Disclaimer 13
6.6. Regulatory Disclaimer 13
6.7. Safety Data Sheet 13
6.8. Contact and Support 13

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	Random Primed DNA Labeling Kit, Control DNA	12.5 µg/ml λDNA.	1 vial, 20 µl
2	Random Primed DNA Labeling Kit, dATP	0.5 mM 2′-deoxyadenosine-5′-triphosphate in 10 mM Tris buffer.	1 vial, 50 µl
3	Random Primed DNA Labeling Kit, dCTP	0.5 mM 2′-deoxycytidine-5′-triphosphate in 10 mM Tris buffer.	1 vial, 50 µl
4	Random Primed DNA Labeling Kit, dGTP	0.5 mM 2′-deoxyguanosine-5′-triphosphate in 10 mM Tris buffer.	1 vial, 50 µl
5	Random Primed DNA Labeling Kit, dTTP	0.5 mM 2′-deoxythymidine-5′-triphosphate in 10 mM Tris buffer.	1 vial, 50 µl
6	Random Primed DNA Labeling Kit, Hexanucleotide mixture	Hexanucleotide mixture in 10x-concentrated reaction buffer.	1 vial, 100 µl
7	Random Primed DNA Labeling Kit, Klenow enzyme	2 U/µl Klenow polymerase, labeling grade in 50% (v/v) glycerol.	1 vial, 50 µl

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / bottle	Label	Storage
1	Control DNA	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
2	dATP	
3	dCTP	
4	dGTP	
5	dTTP	
6	Hexanucleotide mixture	
7	Klenow enzyme	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.*** ⚠ ***Aliquot and store the solution in 2 to 3 vials.***

1.3. Additional Equipment and Reagent required

For labeling with α^{32}P-dCTP

- Heating block or water bath
- Ice bath
- Autoclaved, double-distilled water
- 0.2 M EDTA, pH 8.0
- 20 µCi α^{32}P-dCTP, 3,000 Ci/mmol

For labeling with Digoxigenin-11-dUTP

- Heating block or water bath
- Ice bath
- Autoclaved, double-distilled water
- 0.2 M EDTA, pH 8.0
- Digoxigenin-11-dUTP alkali stable*
- Digoxigenin-11-dUTP alkali labile*
- Biotin-16-dUTP* (optional)

For DNA purification from agarose gels

- High Pure PCR Product Purification Kit*

For removal of non-incorporated radioactivity

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

1.4. Application

The Random Primed DNA Labeling Kit generates probes with high specific activity and can be used in a variety of hybridization techniques:

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

1.5. Preparation Time

Assay Time

50 minutes

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Templates for labeling reaction

- DNA fragments of at least 100 to 200 bp.
- Linearized plasmid or λDNA.
- Minimal amounts of DNA, 10 ng up to 3 µg, such as DNA restriction fragments isolated from gels.
  When using DNA/agarose mixture for labeling after low melting point agarose gel extraction at +100°C, cool down the template to +37°C before adding to the labeling mixture.
- For the standard labeling reaction, use 25 ng DNA.
  Less DNA can also be labeled with the kit, even though maximal incorporation may be achieved only after longer incubation, up to 60 minutes.

General Considerations

Labeling efficiency

The amount of labeled DNA depends on the:
- Amount of template DNA.
- Purity of template DNA.
- Fragment size
- Conformation of template DNA.

Labeling variations

Modified deoxyribonucleoside triphosphates [^{32}P]-, [^{35}S]-, [^{3}H]-, [^{125}I], digoxigenin- or biotin-labeled dUTP can be used in the same way.

Working Solution

Solution	Composition/Preparation	For use in...
Water	Autoclaved, double-distilled water.	Dilution of DNA.
EDTA	0.2 M ethylenediaminetetraacetic acid, pH 8.0.	Stops the reaction.
dNTP stock mix	▪ Prepare a stock mix of unlabeled dNTPS to avoid pipetting mistakes due to low volumes: 1 µl dATP (Vial 2) + 1 µl dGTP (Vial 4) + 1 µl dTTP (Vial 5). ▪ Aliquot dNTP mix and store at −15 to −25°C. ⚠ *Avoid repeated freezing and thawing.*	Labeling mixture with [α-^{32}P] dCTP.
DIG stock mix	▪ Prepare a stock mix to avoid pipetting mistakes due to low volumes: ▪ Mix Digoxigenin-11-dUTP* and dTTP (Vial 5) at a 1:1 ratio. ▪ For each labeling reaction, use 1.6 µl.	Labeling mixture with DIG-11-dUTP.

If a labeled dNTP other than dCTP is used, add 1 µl of dCTP (Vial 3) to the mix instead of the corresponding unlabeled dNTP.

2.2. Protocols

Labeling DNA using α^{32}P-dCTP

See section, **Working Solution** *for additional information on preparing solutions.*
Larger amounts can be labeled by scaling up of all components and volumes.

1. Add to 25 ng linear template DNA, double-distilled water to a final volume of 12 µl in a microfuge tube.
   – For the control reaction, use 2 µl Control DNA (Vial 1) and 7 µl double-distilled water.
2. Denature the DNA by heating in a boiling water bath for 10 minutes at +95°C and chilling quickly in an ice/water bath.
   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 λDNA, use 1 to 2 minutes at +95°C.
3. Centrifuge briefly and add the components in the following order to the freshly denatured probe on ice:

Reagent	Volume [µl]
dNTP stock mix	3
Hexanucleotide mixture (Vial 6)	2
20 µCi α^{32}P-dCTP, 3000 Ci/ mmol, aqueous solution	2
Klenow enzyme (Vial 7)	1

– Mix and centrifuge briefly.
– Incubate at +37°C for 30 minutes.
Longer incubation can increase the yield of labeled DNA.

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

Labeling DNA using Digoxigenin-11-dUTP*

See section, **Working Solution** *for additional information on preparing solutions.*
Larger amounts can be labeled by scaling up of all components and volumes.

1. Add to 25 ng linear template DNA, double-distilled water to a final volume of 12.4 µl in a microfuge tube.
   – For the control reaction, use 2 µl Control DNA (Vial 1) and 10.4 µl double-distilled water.
2. Denature the DNA by heating in a boiling water bath for 10 minutes at +95°C and chilling quickly in an ice/water bath.
   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 λDNA, use 1 to 2 minutes at +95°C.
3. Centrifuge briefly and add the components in the following order to the freshly denatured probe on ice:

Reagent	Volume [µl]
dNTP stock mix	3
DIG stock mix	1.6
Hexanucleotide mixture (Vial 6)	2
Klenow enzyme (Vial 7)	1

– Mix and centrifuge briefly.
– Incubate at +37°C for 1 to 20 hours.
Longer incubation can increase the yield of labeled DNA.

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

Removal of non-incorporated nucleotides

When the labeled DNA is used as hybridization probe, removal of non-incorporated nucleotides is not necessary. However, if you prefer to remove non-incorporated nucleotides, use:

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

2.3. Parameters

Specific Activity

The standard assay routinely yields a specific activity of 1.8 x 10^9 dpm/µg, using different substrate DNAs after 30 minutes of incubation. This corresponds to 65% incorporation.
When varying the ratio of template DNA to labeled dNTP, similar incorporation rates but different levels of specific activity of the labeled probe are obtained.

3. Results

Data analysis

Degree of labeling

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.

Calculation of newly synthesized DNA (ng)

The amount of newly synthesized DNA in the labeling reaction is determined as follows:

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

Calculation of incorporated radioactivity (dpm)

The amount of incorporated radioactivity in dpm is calculated as follows:

$$\mu Ci\ dNTP \times 2.2 \times 10^6 \times \%\ incorporation$$

Specific activity (dpm/µg)

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

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

Typical results

Specific activity and labeling kinetics

Using the Random Primed DNA Labeling Kit, labeling reactions were performed as shown:
- 25 and 100 ng λDNA with 20, 50, and 100 µCi α^{32}P-dCTP, 3,000 Ci/mmol.
- 2,000 ng λDNA with 50 µCi α^{32}P-dCTP, 3,000 Ci/mmol.
- 25 ng λDNA with 50 and 100 µCi α^{32}P-dCTP, 6,000 Ci/mm.

λDNA [ng]	20 µCi	50 µCi	100 µCi	α^{32}P-dCTP, 3,000 Ci/mmol
25	66% 0.9 × 10^9	65% 1.8 × 10^9	62% 2.6 × 10^9	incorporation dpm/µg
100	73% 0.3 × 10^9	71% 0.7 × 10^9	68% 1.1 × 10^9	
2,000	–	53% 0.3 × 10^8	–	

λDNA [ng]	50 µCi	100 µCi	α^{32}P-dCTP, 6,000 Ci/mmol
25	68% 2.3 × 10^9	62% 3.5 × 10^9	incorporation dpm/µg

The reaction kinetics remain similar with 25 ng or 100 ng DNA, see Figures 1 and 2.

The efficiency of the labeling reaction depends on the product used for labeling which may differ in:

- *Sensitivity*
- *Specificity*
- *Half life*
- *Concentration*

Size of labeled fragments

Using the standard assay, size analysis of the radioactive fragments obtained at various time points of incubation with λDNA was performed by denaturing gel electrophoresis; after 10 to 180 minutes incubation, the length of the fragments was 80 to 120 bp on average.

Figure 1

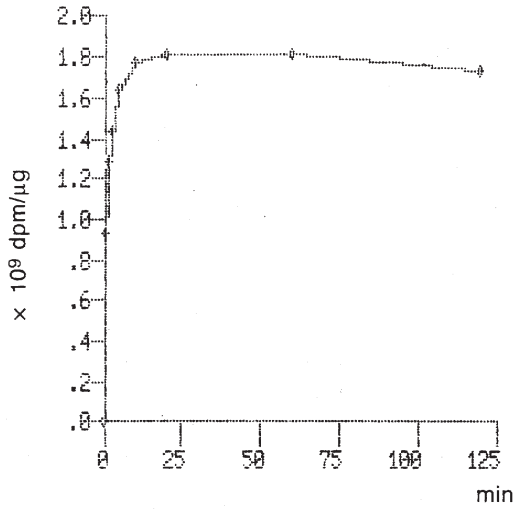

Fig. 1: Labeling kinetics of 25 ng λDNA with 20 µCi α^{32}P-dCTP, 3,000 Ci/mmol.

Figure 2

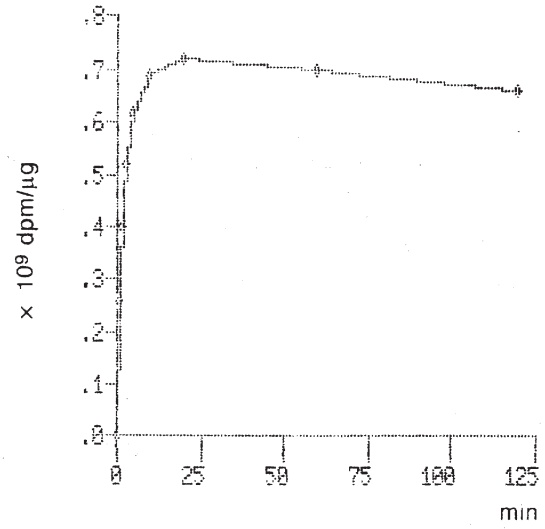

Fig. 2: Labeling kinetics of 100 ng λDNA with 20 µCi α^{32}P-dCTP, 3,000 Ci/mmol.

4. Troubleshooting

Observation	Possible cause	Recommendation
Low amount of synthesized labeled DNA.	Purity of DNA.	Add an additional purification step for template DNA.
	Repeated freezing and thawing damages the nucleotides.	Aliquot the nucleotides and store at −15 to −25°C until use.
	Repeated freezing and thawing damages the Klenow enzyme.	Aliquot Klenow polymerase and store at −15 to −25°C until use.
	Order of pipetting steps was not followed.	It is important to add Klenow polymerase in the last step after adding all other components.
	DNA not fully denatured.	Denaturing time of DNA is crucial. ▪ Use 10 minutes at +95°C, however, depending on the type of DNA, a shortening of the denaturing time down to one minute can effectively improve labeling efficiency. ▪ Quickly chill denatured probe in an ice/water bath to avoid renaturation of template DNA. ▪ Briefly spin down the probe before adding the labeling components. ▪ Pipette uninterrupted once starting the labeling reaction.
	Incorrect incubation temperature.	Incubate DNA at +37°C.
Low specific activity.	Template concentration was too high.	If you use high concentrations of template DNA, the degree of incorporation will increase but the specific activity (dpm/µg) decreases.
	Modified ratio of DNA: labeled NTPs.	When varying ratios of DNA to labeled deoxyribonucleotide-triphosphates are used, similar incorporation rates but different levels of specific activity are obtained.
Inefficient labeling of DNA/agarose mixture.	DNA/agarose mixture for labeling was too hot.	After extraction of DNA from agarose low melting point at +100°C, be sure to cool the template to +37°C before adding to the labeling mixture.

5. Additional Information on this Product

5.1. 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, or fluorescein added to the reaction are readily incorporated into newly synthesized DNA strands.

5.2. Quality Control

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

6. Supplementary Information

6.1. 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.

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Digoxigenin-11-dUTP, alkali-stable	25 nmol, 25 µl, 1 mM	11 093 088 910
	125 nmol, 125 µl, 1 mM	11 558 706 910
	5 x 125 nmol, 5x 125 µl, 1 mM	11 570 013 910
Digoxigenin-11-dUTP, alkali-labile	25 nmol, 25 µl, 1 mM	11 573 152 910
	125 nmol, 125 µl, 1 mM	11 573 179 910
Quick Spin Columns for radiolabeled DNA purification	20 columns	11 273 965 001
	50 columns	11 273 973 001
High Pure PCR Product Purification Kit	1 kit, up to 50 purifications	11 732 668 001
	1 kit, up to 250 purifications	11 732 676 001
Biotin-16-dUTP	50 nmol, 50 µl, 1 mM	11 093 070 910

6.4. Trademarks

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

6.5. License Disclaimer

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

6.6. Regulatory Disclaimer

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

6.7. Safety Data Sheet

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

6.8. 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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