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

Hexanucleotide Mix

Version: 19
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For labeling DNA using random oligonucleotides as primers.

Cat. No. 11 277 081 001 100 µl
10x conc.
50 labeling reactions

Store the product at −15 to −25°C.

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1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	Hexanucleotide Mix, 10x conc.	Reaction buffer: 0.5 M Tris-HCl*, 1 mM dithioerythriol (DTE), 2 mg/ml BSA, hexanucleotides, 62.5 A_{260} U/ml, pH 7.2 at +20°C.	1 vial, 100 µl

The Hexanucleotide Mix, 10x conc. is identical to Vial 6 supplied with the Random Primed DNA Labeling Kit and Vial 5 of the DIG DNA Labeling and Detection Kit.

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	Hexanucleotide Mix, 10x conc.	Store at −15 to −25°C.

1.3. Additional Equipment and Reagent required

For standard labeling

- Heating block or water bath
- 0.2 M EDTA, pH 8.0
- Klenow enzyme, labeling grade*
- Desoxynucleotide-Triphosphate Set*; unlabeled dNTPs are also available as single nucleotides
- Radioactive nucleotides; for radioactive labeling, use α^{32}P-dCTP, 3,000 Ci/mmol

Labeling with Digoxigenin-11-dUTP

- Heating block or water bath
- 0.2 M EDTA, pH 8.0
- DIG DNA Labeling Mix*
- Klenow enzyme, labeling grade*

1.4. Application

Labeled DNA probes with high specific activity are used in a variety of hybridization reactions:

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

2. How to Use this Product

2.1. Before you Begin

Sample Materials

- DNA fragments
- Linearized plasmid DNA
- λDNA

The linearized DNA to be labeled should be purified by phenol/chloroform extraction and ethanol precipitation.

The length of the DNA fragments to be labeled does not influence the reaction. DNA fragments of 100 bp length are labeled equally well as linearized plasmid or λDNA. The input DNA serves solely as a 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) using this method.

Working Solution

Solution	Preparation/Composition	For use in...
Water	Autoclaved, double-distilled water.	Dilution of DNA.
EDTA	0.2 M ethylenediaminetetraacetic acid, pH 8.0.	Stopping the reaction.
dNTP stock mix	dATP, dGTP, and dTTP in a 1:1:1 ratio. *Store aliquots at −15 to −25°C.* ***Avoid repeated freezing and thawing.***	Labeling mixture with [α^{32}P] dCTP.

If a labeled dNTP other than dCTP is used, 1 µl of dCTP must be added to the mix instead of the corresponding unlabeled dNTP.

2.2. Protocols

Labeling DNA using α^{32}P-dCTP

Larger amounts can be labeled by scaling up of all components and volumes.

1. Add to 25 ng template DNA (linear) double-distilled water to a final volume of 9 µl in a microfuge tube.

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.

3. Add the components in the following order to the freshly denatured probe on ice:

Reagent	Volume [µl]
dNTP stock mix	3
Hexanucleotide Mix, 10x conc.	2
50 µCi α^{32}P-dCTP, 3000 Ci/mmol, aqueous solution	5
Klenow enzyme	1

   – Mix and centrifuge briefly.
   – Incubate at +37°C for 30 minutes to overnight.

   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*

Larger amounts can be labeled by scaling up of all components and volumes.
Very small amounts of probe cannot be used to detect rare sequences. Single-copy gene detection requires the entire probe from 300 to 1,000 ng of template.

1. Add to 25 ng template DNA (linear) double-distilled water to a final volume of 15 µl in a microfuge tube.

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.

3. Add the components in the following order to the freshly denatured probe on ice:

Reagent	Volume [µl]
Hexanucleotide Mix, 10x conc.	2
DIG DNA Labeling Mix, 10x conc.	2
Klenow enzyme	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.

3. Results

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.

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 hexanucleotides of all possible sequences to the denatured DNA to be labeled.

- Practically all sequence combinations are represented in the hexanucleotide 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 hexanucleotides used as primers.

② Modified deoxyribonucleoside-triphosphates, such as labeled with ^{32}P, ^{35}S, ^{3}H, digoxigenin, or biotin 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.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Klenow Enzyme	100 U, 2 U/µl	11 008 404 001
	500 U, 2 U/µl	11 008 412 001
DIG DNA Labeling Mix	50 µl, 10x conc., 25 standard reactions	11 277 065 910
Deoxynucleoside Triphosphate Set	4 x 100 µl, 4 x 10 µmol, 4 x 100 mM	11 277 049 001
	40 x 100 µl, 40 x 10 µmol, 40 x 100 mM	11 922 505 001

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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