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DIG DNA Labeling Mix

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For random primed DNA labeling with digoxigenin-dUTP, alkali-labile

Cat. No. 11 277 065 910 50 µl
10x conc.
25 standard reactions

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	DIG DNA Labeling Mix, 10x conc.	dNTP labeling mixture: 1 mM dATP, 1 mM dCTP, 1 mM dGTP, 0.65 mM dTTP, 0.35 mM DIG-dUTP, alkali-labile, pH 7.5 (+20°C).	1 vial, 50 µl

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / Bottle	Label	Storage
1	DIG DNA Labeling Mix, 10x 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

- Water bath
- Ice water
- 0.2 M EDTA, pH 8.0
- Klenow enzyme, labeling grade*
- Hexanucleotide Mix, 10x conc.*
- Autoclaved, double-distilled water

1.4. Application

DIG-labeled probes are used in a variety of hybridization techniques:
- Southern blots
- Northern blots
- Screening of gene libraries
- *In situ* hybridizations

1.5. Preparation Time

Assay Time

Labeling: One hour to overnight.

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 plasmids, cosmid, or λDNA.
- Supercoiled DNA
- Minimal amounts of DNA (10 ng), such as DNA restriction fragments isolated from low-melting point agarose.

2.2. Protocols

Standard Labeling Assay

The steps for the standard labeling assay are shown below.

Larger amounts can be labeled by scaling up of all components and volumes. Linear DNA is labeled more efficiently than circular and supercoiled DNA.

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

2. Add 10 ng to 3 µg DNA and autoclaved, double-distilled water to a final volume of 15 µl.

3. Denature the DNA by heating in a boiling water bath for 10 minutes at +95°C.
   – Chill quickly in an ice water bath.

   Full denaturation is essential for efficient labeling.

4. Add the following 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 for 1 hour to 20 hours (overnight) at +37°C.

   Longer incubation can increase the yield of labeled DNA.

5. Stop the reaction by adding 2 µl 0.2 M EDTA (pH 8.0).

3. Results

Labeling Efficiency

The amount of newly synthesized, labeled DNA depends on the amount and purity of the template DNA. With 1 µg DNA per assay, approximately 10% of the nucleotides are incorporated into about 250 ng of newly synthesized labeled DNA within 1 hour, and approximately 30% of the nucleotides into about 750 ng after 20 hours. Reactions with smaller amounts of template DNA, such as 30 ng, result in a ≥1:1 ratio of labeled to unlabeled DNA (see Table).

Amount of template DNA per labeling reaction [ng]	10	30	100	300	1,000	3,000
Amount of synthesized DIG-labeled DNA [ng] after 1 hour	15	30	60	120	260	530
Amount of synthesized DIG-labeled DNA [ng] after 20 hours	50	120	260	500	780	890

The amount of synthesized, labeled DNA depends on the amount of template DNA in the labeling reaction and on the length of the incubation time at 37°C.

4. Additional Information on this Product

4.1. Test Principle

DIG-labeled DNA probes are generated according to the method of random primed labeling which is based on the hybridization of random oligonucleotides to the denatured DNA template. The complementary DNA strand is synthesized by Klenow enzyme which uses the 3′-OH termini of the random oligonucleotides as primers and a mixture of deoxyribonucleotides containing DIG-11-dUTP, alkali-labile for elongation. DIG dUTP is incorporated every 20 to 25 nucleotides in the newly synthesized DNA. This density of haptens in the DNA yields the highest sensitivity in the detection reaction.

4.2. Quality Control

Function tested with the DIG DNA Labeling Kit* and the DIG Nucleic Acid Detection Kit* (by exchanging the mix for Vial 6 of the DIG DNA Labeling Kit). Using unlabeled control DNA, labeled as described in the protocol, 0.1 pg of homologous DNA are detected in a dot blot after 16 hours color development.

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		
DIG Nucleic Acid Detection Kit	1 kit, Detection of 40 blots of 10 cm x 10 cm	11 175 041 910
Hexanucleotide Mix	100 µl, 10x conc., 50 labeling reactions	11 277 081 001
DIG DNA Labeling Kit	1 kit, 40 labeling reactions of 10 ng to 3 µg DNA	11 175 033 910
Klenow Enzyme	100 U, 2 U/µl	11 008 404 001
	500 U, 2 U/µl	11 008 412 001

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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