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

Digoxigenin-11-dUTP, alkali-labile

Version: 13
Content Version: March 2021

DIG-11-dUTP
Digoxigenin-3-O-methylcarbonyl-ε-aminocaproyl-[5-(3-aminoallyl)-2-deoxy-uridine-5′-triphosphate] tetralithium salt

Cat. No. 11 573 152 910	25 nmol 25 µl, 1 mM
Cat. No. 11 573 179 910	125 nmol 125 µl, 1 mM

Store product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	Digoxigenin-11-dUTP, alkali-labile	1 mM tetralithium salt solution.	11 573 152 910	1 vial, 25 µl
			11 573 179 910	1 vial, 125 µl

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / Bottle	Label	Storage
1	Digoxigenin-11-dUTP, alkali-labile	Store at −15 to −25°C. ⚠ ***A decomposition of approximately 5% may occur within 6 months.***

1.3. Additional Equipment and Reagent required

For random primed DNA labeling reaction

ⓘ *See section,* **Working Solution** *for additional information on how to prepare solutions.*

- Hexanucleotide Mix*
- DIG/dNTP* mixture, 10x conc.
  ⓘ *Also available as a Set of Deoxynucleotides, PCR Grade*.*
- Klenow enzyme*, 100 U
- EDTA, 0.2 M, pH 8.0
- Autoclaved, double-distilled water
- Water bath
- Ice bath

For analysis of PCR products

- PCR DIG Labeling Mix*, or dATP*, dGTP*, dCTP*, dTTP*

For synthesis of probes

- PCR DIG Probe Synthesis Kit*, or dATP*, dGTP*, dCTP*, dTTP*

1.4. Application

Use DIG-11-dUTP, alkali-labile for labeling of probes which are preferentially used in hybridization experiments where stripping and reprobing of the membrane is intended.

DIG-11-dUTP, alkali-labile can be used for the following applications:

- Nonradioactive DNA labeling, such as random priming or nick translation. DIG-11-dUTP replaces dTTP in the random primed DNA labeling reaction or in nick translation in a ratio of 35% DIG-11-dUTP and 65% dTTP.
- Substrate for DNA Polymerase*, Taq DNA Polymerase*, Terminal Transferase*, and Reverse Transcriptase*.

Labeled DNA can be subsequently detected with the:

- DIG Nucleic Acid Detection Kit* or the
- DIG Luminescent Detection Kit for Nucleic Acids*.

For labeling of probes which are preferentially used in experiments where alkaline treatment is required, use DIG-11-dUTP, alkali-stable*.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

DIG-11-dUTP, alkali-labile is used with linearized DNA.

Working Solution

Working solutions for random primed DNA labeling reaction

Reagent/Buffer	Composition/Concentration
DIG/dNTP* mixture, 10x conc.	1 mM dATP
	1 mM dGTP
	1 mM dCTP
	0.65 mM dTTP
	0.35 mM DIG-11-dUTP
	pH 7.5 (+20°C)

2.2. Protocols

Random primed DNA labeling reaction

The following protocol describes a standard assay.

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. Purify the linearized DNA to be labeled by phenol chloroform extraction and ethanol precipitation.

2. To a reaction vial, 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; quickly chill 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/dNTP mixture, 10x conc.	2
Klenow enzyme	1

   – Mix and centrifuge briefly.
   – Incubate for at least 60 minutes at +37°C.

   ⚠ ***Longer incubations up to 20 hours increase the yield of labeled DNA.***

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

Polymerase chain reaction (PCR)

DIG-11-dUTP can be used instead of dTTP as a substrate for Taq DNA Polymerase during PCR. Incorporation of digoxigenin allows the highly sensitive analysis of PCR products or the synthesis of labeled DNA probes. Whereas for the analysis of PCR products, it is sufficient to use a 1:19 ratio of DIG-11-dUTP to dTTP, the DIG-11-dUTP ratio must be increased for highly efficient probe labeling, suitable for single-copy gene detection. Here, use a 2:1 ratio of dTTP to DIG-11-dUTP.

Analysis of PCR products

For a standard PCR setting, use the following nucleotide concentrations: 10 µM DIG-11-dUTP, 190 µM dTTP*, and 200 µM dATP*, dGTP*, dCTP* each. This concentration of labeled nucleotides allows the highly sensitive detection of PCR products after gel electrophoresis and Southern blot or in a microplate-based format.

Alternatively, use the PCR DIG Labeling Mix that contains the required concentration of nucleotides.*

Synthesis of probes

Use the PCR DIG Probe Synthesis Kit* or the following nucleotides: 70 µM DIG-11-dUTP, 130 µM dTTP, and 200 µM dATP, dGTP, dCTP each. Use these probes for single-copy gene detection in Southern blot hybridization with genomic DNA. For a detailed protocol, refer to the Instructions for Use of the PCR DIG Probe Synthesis Kit*.

2.3. Parameters

Chemical Formula

$C_{45}H_{63}N_4O_{22}P_3Li_4$

Chemical Name

Structural formula

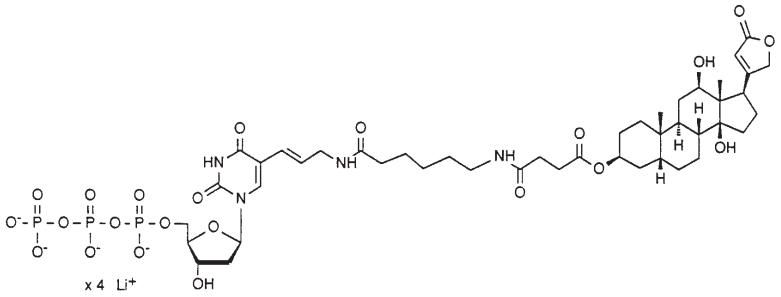

Fig. 1: Chemical structure of DIG-11-dUTP, alkali-labile.

Molecular Weight

1,132.7 Da

3. Supplementary Information

3.1. 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.

3.2. Changes to previous version

Layout changes.
Editorial changes.

3.3. 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
DIG Luminescent Detection Kit	1 kit, 50 blots with a size of 10 x 10 cm^2	11 363 514 910
PCR DIG Probe Synthesis Kit	1 kit, 25 reactions of 50 µl final volume each. One reaction can produce enough labled probe to analyze 650 cm^2 of blot membrane.	11 636 090 910
Taq DNA Polymerase, 1 U/µl	250 U, 1 U/µl, 200 reactions in a final volume of 50 µl	11 647 679 001
	1,000 U, 4 x 250 U, 800 reactions in a final volume of 50 µl	11 647 687 001
Hexanucleotide Mix	100 µl, 10x conc., 50 labeling reactions	11 277 081 001
Deoxynucleoside Triphosphate Set	4 x 250 µl, 4 x 25 µmol, 100 mM	11 969 064 001
	4 x 1,250 µl, 4 x 125 µmol, 100 mM	03 622 614 001
Taq DNA Polymerase, 5 U/µl	100 U, 5 U/µl, 80 reactions	11 146 165 001
	500 U, 5 U/µl, 400 reactions	11 146 173 001
	4 x 250 U, 5 U/µl, 800 reactions	11 418 432 001
	10 x 250 U, 5 U/µl, 2,000 reactions	11 596 594 001
	20 x 250 U, 5 U/µl, 4,000 reactions	11 435 094 001
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
Transcriptor Reverse Transcriptase	250 U, 25 reactions of 20 µl final volume	03 531 317 001
	500 U, 50 reactions of 20 µl final volume	03 531 295 001
	2,000 U, 4 x 500 U, 200 reactions of 20 µl final volume	03 531 287 001
Terminal Transferase	8,000 U, 400 U/µl, 20 tailing or 3'-end labeling reactions (400 U per reaction)	03 333 566 001
	24,000 U, 400 U/µl, 60 tailing or 3'-end labeling reactions (400 U per reaction)	03 333 574 001
PCR DIG Labeling Mix	2 x 250 µl, 2 x 25 PCR assays of 100 µl final volume each	11 585 550 910
Klenow Enzyme	100 U, 2 U/µl	11 008 404 001
	500 U, 2 U/µl	11 008 412 001
dCTP	250 µl, 25 µmol, 100 mM, 6,250 standard PCR assays of 20 µl each.	11 934 520 001
	1,250 µl, 125 µmol, 100 mM, 31,250 standard PCR assays of 20 µl each.	11 969 021 001
	4 x 1,250 µl, 4 x 125 µmol, 100 mM, 125,000 standard PCR assays of 20 µl each.	03 732 690 001
dGTP	250 µl, 25 µmol, 100 mM, 6,250 standard PCR assays of 20 µl each.	11 934 538 001
	1,250 µl, 125 µmol, 100 mM, 31,250 standard PCR assays of 20 µl each.	11 969 030 001
	4 x 1,250 µl, 4 x 125 µmol, 100 mM, 125,000 standard PCR assays of 20 µl each.	03 732 703 001
dTTP	250 µl, 25 µmol, 100 mM, 6,250 standard PCR assays of 20 µl each.	11 934 546 001
	1,250 µl, 125 µmol, 100 mM, 31,250 standard PCR assays of 20 µl each.	11 969 048 001
dATP	250 µl, 25 µmol, 100 mM, 6,250 standard PCR assays of 20 µl each.	11 934 511 001
	1,250 µl, 125 µmol, 100 mM, 31,250 standard PCR assays of 20 µl each.	11 969 013 001
	4 x 1,250 µl, 4 x 125 µmol, 100 mM, 125,000 standard PCR assays of 20 µl each.	03 732 681 001

3.4. Trademarks

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

3.5. License Disclaimer

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

3.6. Regulatory Disclaimer

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

3.7. Safety Data Sheet

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

3.8. 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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