Digoxigenin-11-2'-deoxy-uridine-5'-triphosphate, alkali-labile

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

Cat. No. 11 573 152 910 25 nmol (25 µl)
Cat. No. 11 573 179 910 125 nmol (125 µl)

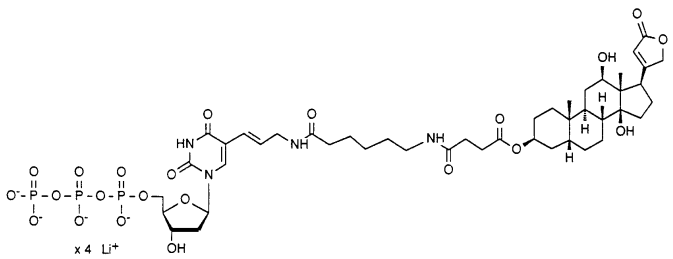
Version Jan. 2006
Store at −15 to −25°C

1. Product Overview

Formulation

1 mM tetralithium salt, solution

Structural Formula



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

Molecular Weight: 1132.7

2. Application

For non-radioactive DNA labeling e.g. random primed or nick translation. The alkali-labile compound should be used for labeling of probes which are preferentially used in hybridization experiments where stripping and reprobing of the membrane is intended. DIG-11-dUTP replaces dTTP in the random primed DNA labeling reaction (1) or in nick translation in a ratio of 35% DIG-11-dUTP and 65% dTTP. It is a substrate for DNA Polymerase, Taq DNA Polymerase, Terminal Transferase (2) 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*.

⚠ Should not be used in experiments where alkaline treatment is required. For this application the use of the alkali-stable DIG-11-dUTP* is recommended.

Sample Material: Linearized DNA

Storage and Stability

The unopened reagent is stable at −15 to −25°C through the expiration date printed on the label.

Decomposition of approx. 5% may occur within 6 months.

* *available from Roche Applied Science*

3. How to Use This Product

3.1 Random Primed DNA Labeling Reaction

Additional Reagents Required

Please refer to the following table.

Reagent/Buffer	Composition/Concentration
Hexanucleotide Mix*	10× conc. hexanucleotides in reaction buffer
DIG/dNTP mixture	10 × concentrated: 1 mM dATP*, 1 mM dGTP*, 1 mM dCTP*, 0.65 mM dTTP*, 0.35 mM DIG-11-dUTP pH 7.5 (20°C) *also available as set
Klenow enzyme*	100 units
EDTA	0.2 M, pH 8.0

Procedure

The following procedure 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.

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

❷ Add 10 ng – 3 µg DNA and autoclaved, double distilled water to a final volume of 15 µl to a reaction vial

❸ Denature the DNA by heating in a boiling water bath for 10 min at 95°C and quickly chilling in an ice/water bath.

🔍 Complete denaturation is essential for efficient labeling.

❹ • Add the following to the freshly denatured probe on ice:

Reagent	Volume
Hexanucleotide Mix, 10 × conc	2 µl
DIG/dNTP mixture	2 µl
Klenow enzyme	1 µl

• Mix and centrifuge briefly.
• Incubate for at least 60 min h at 37°C.

⚠ Longer incubations (up to 20 h) increase the yield of labeled DNA

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

3.2 Polymerase Chain Reaction (PCR) with DIG-11-dUTP

Assay Principle

DIG-11-dUTP can be used instead of dTTP as a substrate for Taq DNA Polymerase during the Polymerase chain reaction (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 ratio of DIG-11-dUTP to dTTP of 1:19, the DIG-11-dUTP ratio has to be increased for highly efficient probe labeling, suitable for single copy gene detection. In this case the ratio of dTTP to DIG-11-dUTP should be 2:1.

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 MTP based format. A PCR DIG labeling mix, that contains the above described concentration of nucleotides is available (see list of Ordering Information).

Synthesis of Probes

We recommend to use our PCR DIG Probe Synthesis Kit* or the following nucleotides:

70 µM DIG-11-dUTP, 130 µM dTTP and 200 µM dATP, dGTP, dCTP each. These probes can be used for single copy gene detection in Southern blot hybridization with genomic DNA. For a detailled protocol please refer to the PCR DIG Probe Synthesis Kit*.

4. Supplementary Information

References

1 Feinberg, A.P. & Vogelstein, B.(1983) *Anal. Biochem.* **132**, 6.

2 Schmitz, G. et al.(1991) *Anal. Biochem.* **193**, 222-231.

Please refer to our website for the following information:

3 DIG Product Selection Guide

4 DIG Application Manual for Filter Hybridization

5 Non-radioactive In situ Hybridization Manual

6 Lab FAQS

Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our homepage http://www.roche-applied-science.com and our Special Interest Sites including:

- DIG Reagents and Kits for Non-Radioactive Nucleic Acid Labeling and Detection: http://www.roche-applied-science.com/DIG/

Product	Pack size	Cat. No.
Enzymes for Labeling		
Klenow enzyme	100 U 500 U	11 008 404 001 11 008 412 001
Taq DNA Polymerase, 1 U/µl	250 U 1000 U (4× 250 U)	11 647 679 001 11 647 687 001
Taq DNA Polymerase, 5 U/µl	100 U	11 146 165 001
Terminal Transferase, recombinant	8 000 U 24 000 U	03 333 566 001 03 333 574 001
Transcriptor Reverse Transcriptase	2000 U (4× 500 U) for 200 reactions 500 U for 50 reactions 250 U for 25 reactions	03 531 287 001 03 531 295 001 03 531 317 001
Nucleotides		
Hexanucleotide Mix	100 µl (50 reactions)	11 277 081 001
Set of Deoxynucleotides, PCR Grade	4× 25 µmol 4× 250 µmol	11 969 064 001 03 622 614 001
DIG Labeling and Detection Reagents		
DIG High Prime Labeling and Detection Starter Kit II	12 labeling reactions and 24 blots	11 585 614 910
DIG Luminescent Detection Kit for Nucleic acids	1 kit (50 blots)	11 363 514 910
DIG Nucleic Acid Detection Kit	40 blots (10× 10 cm)	11 175 041 910
PCR DIG Probe Synthesis Kit	25 reactions	11 636 090 910
PCR DIG Labeling Mix *(for analysis of PCR products)*	2× 250 µl	11 585 550 910
DIG Gel Shift Kit 2nd Generation	1 kit	03 353 591 910
Digoxigenin-11-dUTP, alkali-labile	25 nmol (25 µl) 125 nmol (125 µl)	11 573 152 910 11 57 3179 910
Anti-DIG-AP conjugate, Fab fragments	150 U (200 µl)	11 093 274 910
CDP-*Star*, ready -to-use	2× 50 ml	12 041 677 001
CSPD , ready-to-use	2× 50 ml	11 755 633 001
DIG Easy Hyb (ready-to-use hybridization solution without formamide)	500 ml	11 603 558 001
DIG Wash and Block Buffer Set	30 blots (100 cm^2)	11 585 762 001
NBT/BCIP stock solution	8 ml	11 681 451 001
Nylon Membrane, positively charged	10 sheets (20× 30 cm) 20 sheets (10× 15 cm) 1 roll (0.3× 3 m)	11 209 272 001 11 209 299 001 11 417 240 001
Nylon Membranes for Colony/Plaque Hybridization	50 discs (each 82 mm diameter) 50 discs (each 132 mm diameter)	11 699 075 001 11 699 083 001

Contact and Support

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

www.roche-applied-science.com/support

To call, write, fax, or email us, visit the Roche Applied Science homepage www.roche-applied-science.com and select your home country. Country specific contact information will be displayed. Utilize the Product Search function to get access to Pack Inserts and Material Safety Data Sheets.

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