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

Fluorescein-12-dUTP

Version: 18
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Fluorescein-5(6)-carboxaminocaproyl-[5-(3-aminoallyl)-2′-deoxy-uridine-5′-triphosphate] tetralithium salt

Cat. No. 11 373 242 910 25 nmol
25 µl, 1 mM

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Fluorescein-12-dUTP	1 mM tetralithium salt solution.	1 vial, 25 µl

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	Fluorescein-12-dUTP	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*
- 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

1.4. Application

Fluorescein-12-dUTP can be used for the following applications:

- Nonradioactive DNA labeling, such as random priming, PCR labeling, tailing, or nick translation. Fluorescein-12-dUTP replaces dTTP in the random primed DNA labeling reaction or in nick translation, as well as in the PCR.
- Substrate for DNA Polymerase I (holoenzyme and Klenow fragment)*, Taq DNA Polymerase*, Terminal Transferase (3′-end labeling)*, and Reverse Transcriptase (AMV)*.

ⓘ *Fluorescein-labeled probes can be used for in situ hybridization with direct fluorescence detection.*

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Linearized DNA

Working Solution

Working solutions for random primed DNA labeling reaction

Reagent/Buffer	Composition/Concentration
Fluorescein/dNTP* mixture, 10x conc.	1 mM dATP
	1 mM dGTP
	1 mM dCTP
	0.65 mM dTTP
	0.35 mM Fluorescein-12-dUTP
	pH 7.5 (+20°C)

2.2. Protocols

Random primed DNA labeling reaction

The following protocol describes a labeling reaction using Fluorescein-12-dUTP.

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
Fluorescein/dNTP mixture, 10x conc.	2
Klenow Enzyme (2 U/µl)	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).

2.3. Parameters

Chemical Formula

$C_{39}H_{37}N_4O_{21}P_3Li_4$

Chemical Name

Structural formula

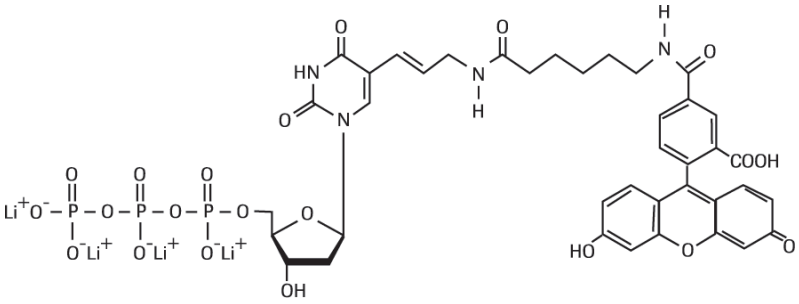

Fig. 1: Chemical structure of Fluorescein-12-dUTP.

Emission

Emission $_{max}$ [nm]: 521 (0.1 M phosphate buffer, pH 9)

Excitation Maximum

Excitation $_{max}$ [nm]: 495

Molecular Weight

1,018.4 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		
Taq DNA Polymerase, 5 U/µl	custom fill, 5 U/µl	11 147 633 103
Transcriptor Reverse Transcriptase	custom fill	03 531 252 103
DNA Polymerase I	250 U	10 642 711 001
	1,000 U	10 642 720 001
Terminal Transferase, recombinant	custom fill	03 289 869 103
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
dATP, PCR Grade	20 ml, 2,000 µmol	04 631 056 103
	100 ml, 10,000 µmol	11 889 516 103
dCTP, PCR Grade	20 mL, 2,000 µmol	04 631 072 103
	100 mL, 10,000 µmol	11 889 508 103
dGTP, PCR Grade	20 ml, 2,000 µmol	04 631 129 103
	100 ml, 10,000 µmol	11 889 524 103
dTTP, PCR Grade	20 mL, 2,000 µmol	04 631 137 103
	100 mL, 10,000 µmol	11 889 559 103
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
Klenow Enzyme	100 U, 2 U/µl	11 008 404 001
	500 U, 2 U/µl	11 008 412 001

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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