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

Tetramethyl-rhodamine-5-dUTP

Version: 11
Content Version: June 2021

Tetramethyl-rhodamine-5-2'-deoxy-uridine-5'-triphosphate

Cat. No. 11 534 378 910 25 nmol
25 µl, 1 mM

Store product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Tetramethyl-rhodamine-5-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	Tetramethyl-rhodamine-5-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*
- Tetramethyl-rhodamine/dNTP* mixture, 10x conc.
  ⓘ *Also available as a Set of Deoxynucleotides, PCR Grade*.*
- Klenow enzyme*, 100 U
- EDTA, 0.2 M, pH 8.0
- High Pure PCR Product Purification Kit*
- Autoclaved, double-distilled water
- Water bath
- Ice bath

1.4. Application

Tetramethyl-rhodamine-5-dUTP is used for nonradioactive labeling of DNA. This modified nucleotide is a substrate for:

- Terminal Transferase*
- DNA Polymerase I * (holoenzyme and Klenow fragment)
- Taq DNA Polymerase*
- Reverse transcriptase, for example, from Transcriptor Reverse Transcriptase* and M-MuLV.

Tetramethyl-rhodamine-5-dUTP can also be used in the following applications:

- Substitute for dTTP in nick-translation reactions and in the random primed labeling techniques for DNA labeling, as well as in the PCR.
- The nucleotide also serves as a substrate for terminal transferase in 3′-end labeling.
- Tetramethylrhodamine-labeled probes show red fluorescence. They are suited for use in *in situ* hybridization for direct fluorescence detection. Multiple fluorescence labeling using Fluorescein-12-dUTP* (yellow fluorescence) or other dye-labeled deoxynucleotides is possible.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Tetramethyl-rhodamine-5-dUTP is used with linearized DNA.

Working Solution

Working solutions for random primed DNA labeling reaction

Reagent/Buffer	Composition/Concentration
Tetramethyl-rhodamine/dNTP* mixture, 10x conc.	1 mM dATP
	1 mM dGTP
	1 mM dCTP
	0.65 mM dTTP
	0.35 mM Tetramethyl-rhodamine-5-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 using the High Pure PCR Product Purification Kit* or 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
Tetramethyl-rhodamine/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

$C_{37}H_{36}N_5O_{18}P_3Li_4$

Chemical Name

Structural formula

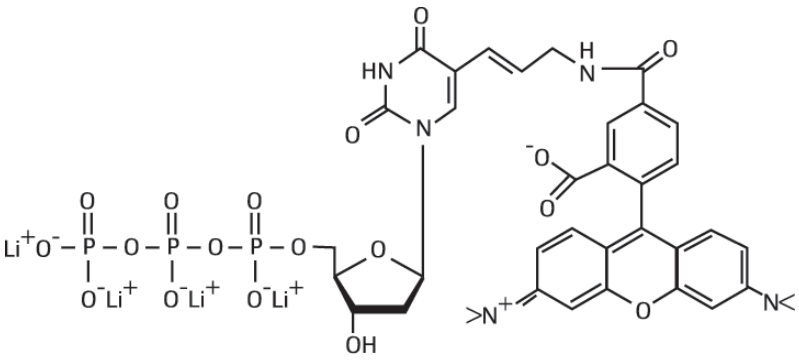

Fig. 1: Chemical structure of Tetramethyl-rhodamine-5-dUTP.

Emission

Emission $_{max}$ [nm]: 575 (0.1 M sodium borate buffer, pH 8.5)

Excitation Maximum

Excitation $_{max}$ [nm]: 551

Molecular Weight

959.4 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		
High Pure PCR Product Purification Kit	1 kit, up to 50 purifications	11 732 668 001
	1 kit, up to 250 purifications	11 732 676 001
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
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
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
Fluorescein-12-dUTP	25 nmol, 25 µl, 1 mM	11 373 242 910
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
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
DNA Polymerase I	250 U	10 642 711 001
	1,000 U	10 642 720 001

Klenow Enzyme	100 U, 2 U/µl	11 008 404 001
	500 U, 2 U/µl	11 008 412 001
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
Hexanucleotide Mix	100 µl, 10x conc., 50 labeling reactions	11 277 081 001
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
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
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

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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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany