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

Fluorescein-12-UTP

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Fluorescein-5(6)-carboxaminocaproyl-[5-(3-aminoallyl)-uridine-5'-triphosphate] tetralithium salt

Cat. No. 11 427 857 910 250 nmol
25 µl, 10 mM

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Fluorescein-12-UTP	10 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-UTP	Store at −15 to −25°C. ⚠ ***A decomposition of approximately 5% may occur within 6 months.***

1.3. Additional Equipment and Reagent required

RNA labeling with Fluorescein-12-UTP

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

- Fluorescein/NTP* mixture, 10x conc.
  ⓘ *Also available as a Ribonucleoside Triphosphate Set*.*
- Transcription Buffer, 10x conc., supplied with RNA polymerases*: 0.4 M Tris-HCl, pH 8.0, 60 mM $MgCl_2$, 100 mM Dithiothreitol (DTT), 20 mM spermidine, 100 mM NaCl
- T7, SP6, or T3 RNA polymerase*, see label for lot-specific value
- Protector RNase Inhibitor*, 40 U/µl in Transcription Buffer with glycerol, 50% (v/v)
- Water, PCR Grade*, (RNase free, autoclaved, double-distilled) or water treated with 0.1% diethylpyrocarbonate (v/v)
- EDTA, 0.2 M, pH 8.0
- Water bath

For analysis of labeled RNA

- Nylon Membranes*
- Anti-Fluorescein-AP, Fab fragments*
- Anti-Fluorescein-POD, Fab fragments*

1.4. Application

Fluorescein-12-UTP can be used in the following applications:
- Substrate for T7, SP6, and T3 RNA polymerases*; replaces UTP in *in vitro* transcription for RNA labeling.
- Linearized template DNA with T7, SP6, or T3 promoter is *in vitro* transcribed with the corresponding RNA polymerases using ATP, GTP, CTP, UTP and Fluorescein-12-UTP, respectively.

Labeled RNA can be used in:
- *In situ* hybridizations and subsequent direct fluorescence detection, or the
- ELISA technique using Anti-Fluorescein-AP, Fab fragments* or Anti-Fluorescein-POD, Fab fragments*.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Fluorescein-12-UTP is used with linearized DNA containing an T7, SP6, or T3 promoter.

Working Solution

Working solutions for RNA labeling reaction with Fluorescein-12-UTP

Reagent/Buffer	Composition/Concentration
Fluorescein/NTP* mixture, 10x conc.	10 mM ATP
	10 mM GTP
	10 mM CTP
	6.5 mM UTP
	3.5 mM Fluorescein-12-UTP
	in Tris-neutralized solution, pH 7.5

2.2. Protocols

RNA labeling by *in vitro* transcription

The following protocol describes a labeling reaction using Fluorescein-12-UTP and SP6, T7, or T3 RNA Polymerases.

1. Add the following to a microcentrifuge tube on ice:

Reagent	Volume [µl]
Linearized template DNA, 1 µg	X
Fluorescein/dNTP mixture, 10x conc.	2
Transcription Buffer, 10x conc.	2
Water, PCR Grade	X
Protector RNase Inhibitor	1
T7, SP6, or T3 RNA polymerase, 40 U	X
Final Volume	**20**

– Mix and centrifuge briefly.
– Incubate for 2 hours at +37°C.
Optional: Remove template DNA by DNase, RNase free-treatment (20 U, 15 minutes, +37°C).*

2. Stop the reaction by adding 2 µl 0.2 M EDTA (pH 8.0) and/or heating to +65°C.

3. Use the labeled probe immediately or store at −15 to −25°C in aliquots.

Analysis of labeled RNA

Analyze the transcript by agarose gel electrophoresis and ethidium bromide staining. Estimate the yield of Fluorescein-labeled RNA from the ratio of DNA to RNA bands, or via spot assay in combination with Nylon Membranes* and direct detection with the Anti-Fluorescein-AP, Fab fragments* or Anti-Fluorescein-POD, Fab fragments* and appropriate substrates.

Labeling efficiency

Depending on the length, purity, and sequence of the template DNA, approximately 10 µg of Fluorescein-labeled RNA are synthesized under standard conditions. An average of 10 µg RNA per 1 µg template DNA is obtained.

2.3. Parameters

Chemical Formula

$C_{39}H_{37}N_4O_{22}P_3Li_4$

Chemical Name

Structural formula

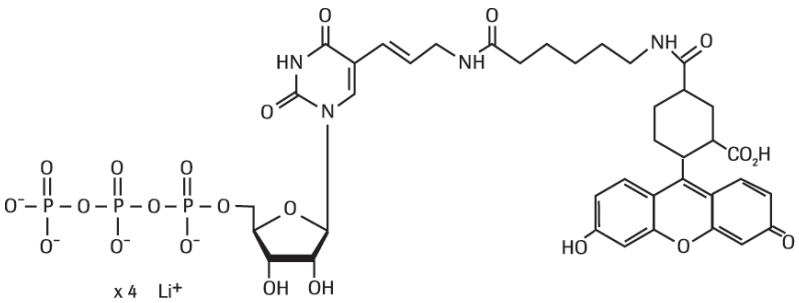

Fig. 1: Chemical structure of Fluorescein-12-UTP.

Emission

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

Excitation Maximum

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

Molecular Weight

1,034.4 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.
Units updated for Protector RNase Inhibitor.

3.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
SP6 RNA Polymerase	1,000 U, > 20 U/µl	10 810 274 001
	5,000 U, > 20 U/µl	11 487 671 001
GTP	400 µl, 40 µmol, 100 mM	11 140 957 001
Protector RNase Inhibitor	2,000 U, 40 U/µl	03 335 399 001
	10,000 U, 5 x 2,000 U	03 335 402 001
ATP	400 µl, 100 mM 40 µmol	11 140 965 001
Anti-Fluorescein, Fab fragments	Anti-Fluorescein-AP, Fab fragments, 150 U, 200 µl	11 426 338 910
	Anti-Fluorescein-POD, Fab fragments, 150 U	11 426 346 910
UTP	400 µl, 40 µmol, 100 mM	11 140 949 001
Anti-Fluorescein-POD, Fab fragments	Anti-Fluorescein-POD, Fab fragments, 150 U	11 426 346 910
T3 RNA Polymerase	1,000 U, ≥ 20 U/µl	11 031 163 001
	5,000 U, ≥ 20 U/µl	11 031 171 001
Ribonucleoside Triphosphate Set	4 x 200 µl, 4 x 20 µmol, 100 mM each	11 277 057 001
DNase I recombinant, RNase-free	10,000 U, 10 U/µl	04 716 728 001
CTP	400 µl, 40 µmol, 100 mM	11 140 922 001
T7 RNA Polymerase	1,000 U, ≥ 20 U/µl	10 881 767 001
	5,000 U, ≥ 20 U/µl	10 881 775 001
Nylon Membranes, positively charged	10 sheets, 20 x 30 cm	11 209 272 001
	20 sheets, 10 x 15 cm	11 209 299 001
	1 roll, 0.3 x 3 m	11 417 240 001
Water, PCR Grade	25 ml, 25 x 1 ml	03 315 932 001
	25 ml, 1 x 25 ml	03 315 959 001
	100 ml, 4 x 25 ml	03 315 843 001

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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