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

Digoxigenin-11-UTP

Version: 17
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DIG-11-UTP
Digoxigenin-3-O-methylcarbonyl-ε-aminocaproyl-[5-(3-aminoallyl)-uridine-5′-triphosphate]
tetralithium salt

Cat. No. 11 209 256 910	250 nmol 25 µl, 10 mM
Cat. No. 03 359 247 910	200 nmol 57 µl, 3.5 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-UTP	10 mM tetralithium salt solution.	11 209 256 910	1 vial, 25 µl
		3.5 mM tetralithium salt solution.	03 359 247 910	1 vial, 57 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-UTP	Store at −15 to −25°C. ⚠ ***A decomposition of approximately 5% may occur within 6 months.***

1.3. Additional Equipment and Reagent required

For RNA labeling with DIG-11-UTP

- DIG/NTP* mixture, 10x conc.
  ℹ *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
- 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, sterile, 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

- DIG-labeled Control RNA*
- CDP-*Star**
- CSPD, ready-to-use*

1.4. Application

DIG-11-UTP can be used in the following applications:
- Substrate for T7, SP6, and T3 RNA polymerases*; replaces UTP in *in vitro* transcription for DIG-labeling of RNA in a ratio of 35:65%.
- Linearized template DNA with T7, SP6, or T3 promoter is *in vitro* transcribed with the corresponding RNA polymerases using ATP, GTP, CTP, UTP, and DIG-11-UTP, respectively.

Labeled RNA can be subsequently detected with the:
- DIG Luminescent Detection Kit for Nucleic Acids* or the
- Anti-Digoxigenin-AP, Fab fragments* and CDP-*Star**.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

DIG-11-UTP is used with linearized DNA containing an T7, SP6, or T3 promoter.

The amount of synthesized labeled RNA depends on the amount, size (site of linearization), and purity of the template DNA.

Avoid RNase contamination: After restriction digest, purify the linearized DNA with the High Pure PCR Product Purification Kit* or via phenol/chloroform extraction, and subsequent ethanol precipitation.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Working solutions for RNA labeling reaction with DIG-11-UTP

Reagent/Buffer	Composition/Concentration
DIG/NTP* mixture, 10x conc.	10 mM ATP
	10 mM GTP
	10 mM CTP
	6.5 mM UTP
	3.5 mM DIG-11-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 DIG-11-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
DIG/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 DIG-labeled RNA by comparison to DIG-labeled control RNA* in a spot test with chemiluminescent detection (CDP-*Star*® or CSPD, ready-to-use®).

Labeling efficiency

Depending on the length, purity, and sequence of the template DNA, approximately 10 µg of DIG-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

$C_{43}H_{61}N_4O_{22}P_3Li_4$

Chemical Name

Structural formula

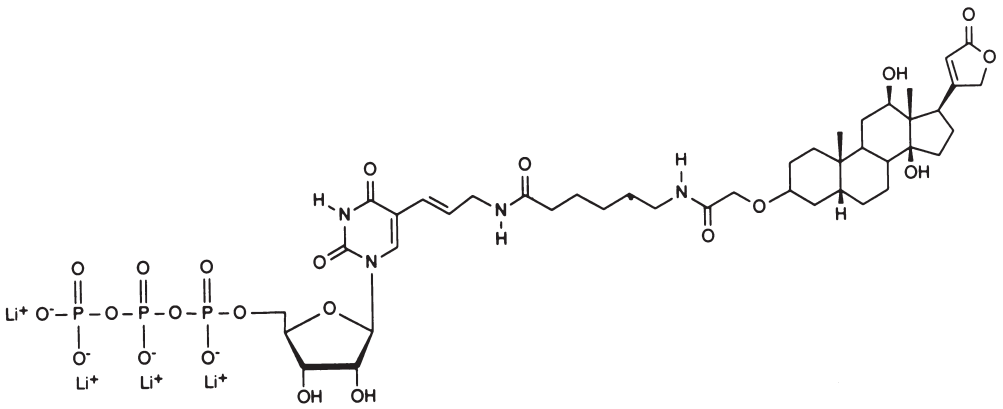

Fig. 1: Chemical structure of Digoxigenin-11-UTP.

Molecular Weight

1,106.7 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.
Update to include new safety Information to ensure handling according controlled conditions.

3.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
T7 RNA Polymerase	1,000 U, ≥ 20 U/µl	10 881 767 001
	5,000 U, ≥ 20 U/µl	10 881 775 001
CSPD, ready-to-use	2 x 50 ml	11 755 633 001
DIG-labeled Control RNA	50 µl, 100 µg/ml DIG-labeled RNA	11 585 746 910
CDP-*Star*, ready-to-use	2 x 50 ml	12 041 677 001
DNase I recombinant, RNase-free	10,000 U, 10 U/µl	04 716 728 001
DIG Luminescent Detection Kit	1 kit, 50 blots with a size of 10 x 10 cm^2	11 363 514 910
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
SP6 RNA Polymerase	1,000 U, > 20 U/µl	10 810 274 001
	5,000 U, > 20 U/µl	11 487 671 001
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
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
Anti-Digoxigenin-AP, Fab fragments	150 U, 200 µl	11 093 274 910
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
CTP	400 µl, 40 µmol, 100 mM	11 140 922 001
UTP	400 µl, 40 µmol, 100 mM	11 140 949 001
ATP	400 µl, 100 mM 40 µmol	11 140 965 001

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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