Biotin-11-CTP

Biotin-ε- aminocaproyl-[5-(3-aminoallyl)-cytidine-5'-triphosphate] disodium salt

Cat. No. 04 739 205 001
250 nmol (25 µl) 10 mM

Version 04
Content version: August 2018
Store at − 15 to − 25°C

1. What this Product Does

Contents

10 mM solution

Storage and Stability

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

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

Application

The preparation is used as substrate for T7, SP6 and T3 RNA polymerases and for RNA labeling to replace CTP in *in vitro* transcription.

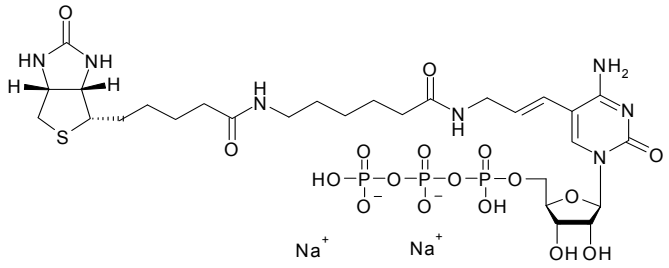
Double-stranded DNA (i.e. linearized plasmid or cDNA) containing a T7, SP6, or T3 promoter serves as template for *in vitro* transcription with the corresponding RNA polymerase using ATP, GTP, CTP, UTP and Biotin-11-CTP. For a higher and/or more uniform label density Biotin-11-CTP can be used together with Biotin-16-UTP in the *in vitro* transcription reaction.

Labeled RNA can be subsequently detected with a fluorescent streptavidin conjugate or by ELISA using a streptavidin-AP conjugate

Product Characteristics

Formular	$C_{28}H_{44}N_7Na_2O_{17}P_3S$
Molecular weight	921.66

Structural Formula



2. How to Use this Product

2.1 Before You Begin

Additional Equipment and Reagents Required

RNA labeling for microarray applications using ds cDNA as template

- Biotin-16-UTP* (optional), 10 mM

RNA labeling using linearized plasmid DNA as template

- NTPs*: ATP, GTP, CTP, UTP
- Transcription buffer (is supplied with RNA polymerases)
- 10× conc.: 0.4 M Tris-HCl, pH 8.0, 60 mM $MgCl_2$, 100 mM dithiothreitol (DTT), 20 mM spermidine, 100 mM NaCl
- T7, SP6, T3 RNA polymerase*
- Protector RNase Inhibitor*
- Water, PCR Grade*; alternatively, water treated with 0.1% diethylpyrocarbonate (v/v) can be used.
- EDTA, 0.2 M, pH 8.0

Sample Material

Linearized plasmid DNA or double-stranded cDNA (ds cDNA) containing a 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 plasmid DNA with the High Pure PCR Product Purification Kit*, or via phenol/chloroform extraction, and subsequent ethanol precipitation.

2.2 Protocol

RNA labeling with Biotin-11-CTP by *in vitro* transcription with T7 RNA polymerase* (control reaction; components are included in the Microarray RNA Target Synthesis Kit (T7)*

❶ Add the following to a microcentrifuge tube at +15 to +25°C:

Component	Volume	Final conc.
Control DNA pSPT18neo, linearized	1.5 µl	
NTP mix (25 mM ATP, 25 mM GTP, 25 mM UTP, 18.75 mM CTP)	4 µl	5 mM ATP 5 mM GTP 5 mM UTP 3.75 mM CTP
Biotin-11-CTP (10 mM)	2.5 µl	1.25 mM (1:4 label densitiy)
Transcription buffer, 10× conc.	2 µl	1 ×
DTT	2 µl	10 mM
H2O, sterile, RNase free	5 µl	
Transcription enzyme blend	3 µl	
Final volume	20 µl	

❷ • Mix and centrifuge briefly.
• Incubate for 2-3 h at +37°C.

❸ We recommend to purify the labeled RNA immediately with the High Pure PCR Product Purification Kit* to stop the reaction:

❹ After purification, use the labeled RNA immediately or store aliquots at -80°C.

2.3 Analysis of labeled RNA

The transcript can be analyzed by agarose-gel electrophoresis and ethidium bromide staining.

Prior to usage as a hybridization probe we recommend to estimate the yield of Biotin-labeled RNA via spot assay in combination with nylon membrane* and detection with the streptavidin-alkaline phosphatase conjugate*.

2.4 Labeling efficiency

The yield of labeled cRNA depends on the amount of template used as well as on the reaction time. The labeling density depends on the nature of the DNA template and on the concentration of labeled nucleotides.

3. Additional Information on this Product

3.1 Reference

1 Lassonczyk, N. et al., (2005) Biochemica, 4, 15-16.

4. Supplementary Information

4.1 Text Conventions

To make information consistent and memorable, the following text conventions are used in this document:

Text conventions	Use
Numbered Instructions labeled ①, ②, etc.	Steps in a process that usually occur in the order listed
Numbered Instructions labeled ❶, ❷, etc.	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Diagnostics

Symbols

In this document the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

4.2 Changes to previous version

- Editorial changes.

4.3 Ordering Information

	Product	Pack size	Cat. No
Kits	High Pure PCR Product Purification Kit	1 kit (50 purifications)	11 732 668 001
	SP6/T7 Transcription Kit	1 kit (2 × 20 reactions)	10 999 644 001
Single Reagent	Streptavidin-AP	150 U (200 µl)	11 093 266 910
	ATP	40 µmol (400 µl)	11 140 965 001
	Biotin RNA Labeling Mix	40 µl (20 reactions)	11 685 597 910
	Biotin-16-UTP	250 nmol (25 µl)	11 388 908 910
	CTP	40 µmol (400 µl)	11 140 922 001
	GTP	40 µmol (400 µl)	11 140 957 001
	Hybridization Bags	50 bags	11 666 649 001
	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
	Ribunucleoside triphosphate Set	1 set 4 × 20 µmol (4 × 200 µl)	11 277 057 001
	Protector RNase Inhibitor	10 000 U 2 000 U	03 335 402 001 03 335 399 001
	T7 RNA Polymerase	1000 U 5000 U	10 881 767 001 10 881 775 001
	T3 RNA Polymerase	1000 U 5000 U	11 031 163 001 11 031 171 001
	SP6 RNA Polymerase	1000 U 5000 U	10 810 274 001 11 487 671 001
	UTP	40 µmol (400 µl)	11 140 949 001

Contact and Support

To ask questions, solve problems, suggest enhancements and 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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