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DATA SHEET

Version: 02
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1. Identification

Product name

T7 Biotin16 RNA Labeling Kit

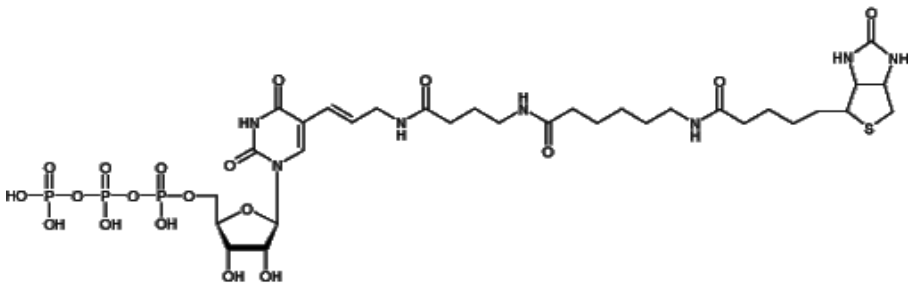
RNA Synthesis with Modified Nucleotides
30 reactions

Cat. No. **RS026**

2. Description

Biotin-16-UTP (Biotin-16-uridine-5'-triphosphate) is a biologically active analog of UTP (uridine-5'-triphosphate) that is readily incorporated into RNA during an in vitro transcription reaction by RNA polymerases such as phage T7 RNA Polymerase.

Since T7 RNA polymerase does not significantly discriminate between the natural and the biotinylated nucleotide, the number of biotinylated positions per RNA transcript can be controlled by adjusting the ratio of the biotinylated nucleoside triphosphate (NTP) to the naturally occurring NTP in the transcription reaction. The biotin-labeled RNA can then be detected by a reporter molecule attached to streptavidin, avidin, or anti-biotin antibody.



T7 Biotin16 RNA Labeling Kit is designed to generate randomly biotin-modified RNA probes by in vitro transcription. Such probes are suited for in situ hybridization and Northern blot hybridization experiments.

3. Kit components

Item	Amount
T7 RNA Polymerase mix *	2 x 40 µl
T7 10X Reaction Buffer **	200 µl
ATP (100 mM)	100 µl
GTP (100 mM)	100 µl
CTP (100 mM)	100 µl
UTP (100 mM)	100 µl
Biotin-16-UTP (10mM)	30 µl
DTT (100 mM)	150 µl
Control template 1 (200 ng/µl)	10 µl
Nuclease-Free Water	1.2 ml

*Include RNase inhibitor

**HEPES-based

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4. Storage specifications

Store all kit components at -20 ˚C. Avoid freeze/thaw cycles.
T7 Biotin16 RNA Labeling Kit components are stable up to the expiry date specified with the product.

5. Applications

- In situ hybridization
- Southern blots
- Microarray hybridization
- Chromosome mapping

6. Further information

Product Use Limitations This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Disclaimer The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. Canvax Reagents S.L.U. shall not be held liable for any damage resulting from handeling or from contact with the above product.

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IN VITRO TRANSCRIPTION PROTOCOL

Materials to Be Supplied by the User:

- T7 Promotor-containing DNA template
- RNA purification tools
- RNAse-free DNAse I

Important Notes before starting:

- Take all precautions to avoid RNase contamination during the whole process. We strongly recommend wearing gloves and using nuclease-free tubes and reagents to avoid RNase contamination.
- Reactions are typically 20 µL but can be scaled up as needed.
- All reagents should be spin briefly before opening to prevent loss and/or contamination.
- The quality and purity of input RNA and resulting cDNA determine the yield of biotin-labeled RNA.
- The recommended molar ratio of Biotin-16-UTP to standard NTP is 1:3 or 1:2.
- The kit contains sufficient reagents for 30 labeling reactions of 20 µl each (35 % Biotin-16-UTP substitution, 1 mM ATP, GTP, CTP, 0.65 mM UTP, 0.35 mM Biotin-16-UTP).

Preparation of working solutions:

A. Preparation of 10 mM ATP/CTP/GTP working solution

- Thaw 100 mM ATP, 100 mM CTP and 100 mM GTP solutions on ice, vortex and spin-down briefly.
- Prepare a 1:10 dilution with Nuclease-Free Water to achieve a final concentration of 10 mM (e.g. 5 µl 100 mM ATP + 5 µl 100 mM CTP + 5 µl 100 mM GTP + 35 µl Nuclease-Free Water).
- 10 mM ATP/CTP/GTP working solution can be stored at -20˚C. Avoid freeze/thaw cycles.

B. Preparation of 10 mM UTP working solution

- Thaw 100 mM UTP solution on ice, vortex and spin-down briefly.
- Prepare a 1:10 dilution with Nuclease-Free Water to achieve a final concentration of 10 mM (e.g. 5 µl 100 mM UTP + 45 µl Nuclease-Free Water).
- 10 mM UTP working solution can be stored at -20 ˚C. Avoid freeze/thaw cycles.

Transcription Reaction Setup (20µl):

1. Place T7 RNA Polymerase Mix on ice.
2. Thaw all remaining components at room temperature (RT), mix and pulse-spin in microfuge to collect solutions to bottom of tubes.
3. Assemble the reaction at room temperature in the following order:

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Component	Volume	Final conc.
1. Nuclease-Free Water	X µl	
2. T7 10X Reaction Buffer (10x)	2 µl	1x
3. DTT (100 mM)	2 µl	10 mM
4. 10 mM ATP/CTP/GTP	2 µl	1 mM
5. 10 mM UTP	1.3 µl	0.65 mM
6. 10 mM Biotin-16-UTP	0.7 µl	0.35 mM
7. Template DNA	X µl	0.5-1 µg
8. T7 RNA Polymerase Mix	2 µl	

4. Mix thoroughly, pulse-spin in microfuge. Incubate at 37˚C for 30 minutes in the dark.
   Depending on the RNA probe individual optimization may increase product yield (2h – 4h at 37 ˚C).
   Note that, modified ribonucleotides reduce transcription efficiency; therefore, lower transcription yields should be expected as compared to transcription using unmodified NTP.
5. **Optional:** Once the in vitro transcription reaction is finished, treat the sample with RNase-Free DNase I to remove template DNA and purify the RNA. *For many applications it may not be necessary.*
   Proceed with a DNase digestion as following:
   Add RNase-free DNase I (not provided) plus the respective reaction buffer directly to the transcription reaction; mix and incubate for 15 minutes at 37 ˚C. Stop DNase activity by adding 1 µL of EDTA at 0.5 M.
6. Proceed with purification of synthesized RNA or analysis of transcription products by gel electrophoresis.