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

Biotin RNA Labeling Mix

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RNA labeling with biotin-16-UTP by *in vitro* transcription with SP6, T7, and T3 RNA polymerases.

Cat. No. 11 685 597 910 40 µl
20 transcription reactions

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Biotin RNA Labeling Mix, 10x conc.	NTP labeling mixture: 10 mM ATP, 10 mM CTP, 10 mM GTP, 6.5 mM UTP, 3.5 mM Biotin-16-UTP, pH 7.5 (+20°C)	1 vial, 40 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	Biotin RNA Labeling Mix, 10x conc.	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.*** ⚠ ***To avoid contamination, aliquot and store the solution in 2 to 3 vials.***

1.3. Additional Equipment and Reagent required

For standard labeling assay

ⓘ *See section,* **Working Solution** *for additional information on preparing solutions.*

- T7, SP6, or T3 RNA Polymerase*
- DNase I recombinant, RNase-free* (optional)
- Transcription buffer 10x conc.

ⓘ *Also supplied with the RNA polymerases**

- RNase-free, autoclaved, double-distilled water, or Water, PCR Grade*
- 0.2 M EDTA, pH 8.0
- Water bath

1.4. Application

Biotin-labeled RNA is used in a variety of hybridization techniques:

- Northern blots
- Southern blots
- Plaque or colony lifts
- RNase protection experiments
- *In situ* hybridization

ⓘ *The Biotin RNA Labeling Mix is designed for use with SP6, T7, and T3 RNA Polymerases* which are supplied with an optimized transcription buffer.*

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Templates for labeling reaction

Linearized plasmid DNA

- The DNA to be transcribed is cloned into the polylinker site of an appropriate transcription vector which contains adjacent to the polylinker, a promoter for SP6, T7, or T3 RNA polymerase.
- For the synthesis of “run off” transcripts, the plasmid is linearized by a restriction enzyme. Use restriction enzymes creating 5′ overhangs; avoid 3′ overhangs. Purify the linearized template DNA by phenol/chloroform extraction and ethanol precipitation to avoid RNase contamination. Use circular plasmid DNA for “run around” transcription.

PCR product

- PCR fragments which contain RNA polymerase promoter sequences can also act as templates for transcription. Purify the correct PCR fragment by gel electrophoresis prior to transcription.

General Considerations

DNase treatment

When the biotin-labeled RNA is used for hybridization to northern or Southern blots, or plaque or colony lifts, a DNase-treatment is not required since the amount of biotin-labeled RNA transcript is far in excess of the template DNA.

Analysis of labeled RNA

Quality and quantity of the transcript can be analyzed by non-denaturing agarose gel electrophoresis and ethidium bromide staining. The signal from the RNA band should be stronger than that from the DNA. The size and the amount of the transcript can be estimated by comparison to known RNAs.

Hybridization with labeled RNA

Add 0.2 to 1 µl (approximately 20 to 100 ng) of the biotin-labeled RNA per ml hybridization solution.

Detection of biotin-labeled RNA probes

After hybridization to nucleic acid targets bound to Nylon Membranes*, the biotin label is detected by an immunoassay with Streptavidin-AP conjugate* and the color substrates NBT/BCIP* or the chemiluminescent substrates CSPD* or CDP-*Star**. For *in situ* hybridizations, avidin conjugated to fluorophores is used.

Working Solution

Solution	Composition
Transcription buffer, 10x conc.	400 mM Tris-HCl*, pH 8.0 (+20°C), 60 mM $MgCl_2$, 100 mM Dithiothreitol (DTT)*, 20 mM spermidine
EDTA	0.2 M ethylenediaminetetraacetic acid, pH 8.0

2.2. Protocols

Standard labeling assay

The steps for the standard labeling assay are shown below.
⚠ ***Work under RNase-free conditions at all times.***

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

Reagent	Volume [µl]
1 µg linearized plasmid DNA, or appropriate amount of PCR product (100 to 200 ng).	X
Biotin RNA Labeling Mix, 10x conc.	2
Transcription buffer, 10x conc.	2
Add autoclaved, RNase-free, double-distilled water to a final volume of 18 µl.	X
T7, SP6, or T3 RNA Polymerase	2
Final Volume	**20**

– Mix and centrifuge briefly.
– Incubate for 2 hours at +37°C.

2. Remove template DNA by adding 2 µl DNase I recombinant, RNase-free* for 15 minutes at +37°C.
i This is an optional step only required for RNase-protection experiments.

3. Stop the reaction by adding 2 µl 0.2 M EDTA, pH 8.0.

4. Use the labeled probe immediately or store ethanol precipitated at −15 to −25°C or −60°C or below.

3. Results

Labeling efficiency

In the standard reaction, approximately 10 µg full-length biotin-labeled RNA is synthesized from 1 µg linearized plasmid DNA with an insert of approximately 1 kb. Larger amounts of biotin-labeled RNA can be obtained by scaling up the reaction components. The amount of synthesized labeled RNA depends on the amount, size (site of linearization), and purity of the template DNA.

Longer incubations do not increase the yield of labeled RNA.

4. Additional Information on this Product

4.1. Test Principle

Biotin-16-UTP is incorporated by SP6, T7, and T3 RNA polymerases at approximately every 20 to 25th nucleotide of the transcript under the conditions described.

5. Supplementary Information

5.1. 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.

5.2. Changes to previous version

Layout changes.
Editorial changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
NBT/BCIP Ready-to-Use Tablets	20 tablets	11 697 471 001
Nylon Membranes for Colony and Plaque Hybridization	50 discs, 82 mm diameter	11 699 075 001
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
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
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
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
Streptavidin Conjugates	Streptavidin-AP Conjugate, 1,000 U	11 089 161 001
	Streptavidin-β-Gal Conjugate, 500 U, *Not available in US*	11 112 481 001
	Streptavidin-POD Conjugate, 500 U	11 089 153 001
1,4-Dithiothreitol	2 g	10 197 777 001
	10 g	10 708 984 001
	25 g	11 583 786 001

5.4. Trademarks

All product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. 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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