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

Fluorescein RNA Labeling Mix

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For RNA labeling with fluorescein-12-UTP by *in vitro* transcription with SP6, T7, and T3 RNA polymerases.

Cat. No. 11 685 619 910 40 µl
10x conc.
20 reactions

Store the product at −15 to −25°C.

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1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	Fluorescein RNA labeling Mix, 10x conc.	NTP labeling mixture: 10 mM ATP, 10 mM CTP, 10 mM GTP, 6.5 mM UTP, 3.5 mM Fluorescein-12-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	Fluorescein 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 the labeling assay

- 0.2 M EDTA, pH 8.0
- Autoclaved, RNase-free, double-distilled water
- SP6 RNA Polymerase*, or
- T7 RNA Polymerase*, or
- T3 RNA Polymerase*
- DNase I, RNase-free* (optional)
- Transcription buffer, 10x conc. is supplied with the RNA polymerases: 400 mM Tris-HCl, pH 8.0 (+20°C), 60 mM $MgCl_2$, 100 mM dithiotreitol (DTT), 20 mM spermidine.

For detection of fluorescein-labeled RNA

- Nylon membranes*
- Anti-Fluorescein-AP, Fab fragments*
- NBT/BCIP*, or
- CSPD* or CDP-*Star**

1.4. Application

Fluorescein-labeled RNA probes are used in a variety of hybridization techniques:

- Southern blots
- Plaque or colony lifts
- *In situ* hybridizations to tissues
- RNase protection experiments

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Templates for labeling reaction

Linearized plasmid DNA

The DNA chosen for transcription 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; 3′ overhangs should be avoided.
- Purify the linearized template DNA by phenol chloroform extraction and ethanol precipitation to avoid RNase contamination.
- For run-around transcription, use circular plasmid DNA.

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

Analysis of labeled RNA

Quality and quantity of the transcript can be analyzed by nondenaturing 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 fluorescein-labeled RNA per ml hybridization solution for membrane blots.

Detection of fluorescein- labeled RNA

After hybridization to nucleic acid targets bound to nylon membrane, the fluorescein label is detected by an immunoassay, for example, with Anti-Fluorescein-AP conjugate* diluted 1:10,000 and the color substrate NBT/BCIP*, or the chemiluminescent substrates CSPD* or CDP-*Star**. Detailed detection protocols are available in the Instructions for Use of the alkaline phosphatase substrates.

DNase treatment

When the fluorescein-labeled RNA is used for hybridization to Southern blots, plaque or colony lifts, or in *in situ* hybridizations, a DNase-treatment is not required, as the amount of fluorescein-labeled RNA transcripts is far in excess of the template DNA.

2.2. Protocols

Standard labeling assay

The steps for the standard labeling assay are shown below.
⚠ ***Always work under RNase-free conditions.***

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

Reagent	Volume [µ]
1 µg linearized plasmid DNA, or appropriate amount of PCR product (100 – 200 ng)	X
Fluorescein RNA Labeling Mix, 10x conc.	2
Transcription buffer, 10x conc.	2
Autoclaved, RNase-free, double-distilled water	add up to 18
RNA Polymerase* (SP6, T7, or T3)	2
Final volume	**18**

2. Mix and centrifuge briefly.
3. Incubate for 2 hours at +37°C.
4. Add 2 µl DNase I, RNase-free to remove template DNA (optional).
   – Incubate for 15 minutes at +37C.
   ⚠ ***Only required for RNase-protection experiments.***
5. Stop the reaction by adding 2 µl of 0.2 M EDTA, pH 8.0.
6. Use the labeled probe immediately or store ethanol-precipitated at −20 or −60°C or below.

3. Results

Labeling efficiency

In the standard reaction, approximately 10 µg full-length fluorescein-labeled RNA is synthesized from 1 µg linearized plasmid DNA with an insert of approximately 1 kb.

- Larger amounts of fluorescein-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.
  ℹ *Longer incubations do not increase the yield of labeled RNA.*

4. Additional Information on this Product

4.1. Test Principle

Fluorescein-labeled, single-stranded RNA probes of defined length are generated by *in vitro* transcription.

- Fluorescein-12-UTP is incorporated by RNA polymerases at approximately every 20 to 25th nucleotide of the transcript under standard conditions.
- The Fluorescein RNA Labeling Mix is specifically designed for use with SP6, T7, and T3 RNA Polymerases* which are supplied with an optimized transcription buffer.

4.2. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

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.	
①②③ etc.	Stages in a process that usually occur in the order listed.
❶❷❸ 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		
SP6 RNA Polymerase	1,000 U, > 20 U/µl	10 810 274 001
	5,000 U, > 20 U/µl	11 487 671 001
T7 RNA Polymerase	1,000 U, ≥ 20 U/µl	10 881 767 001
	5,000 U, ≥ 20 U/µl	10 881 775 001
T3 RNA Polymerase	1,000 U, ≥ 20 U/µl	11 031 163 001
	5,000 U, ≥ 20 U/µl	11 031 171 001
DNase I recombinant, RNase-free	10,000 U, 10 U/µl	04 716 728 001
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
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
NBT/BCIP Stock Solution	8 ml	11 681 451 001
NBT/BCIP Ready-to-Use Tablets	20 tablets	11 697 471 001
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

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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