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DATA SHEET

Version: 02
Revision date: 13/03/2023

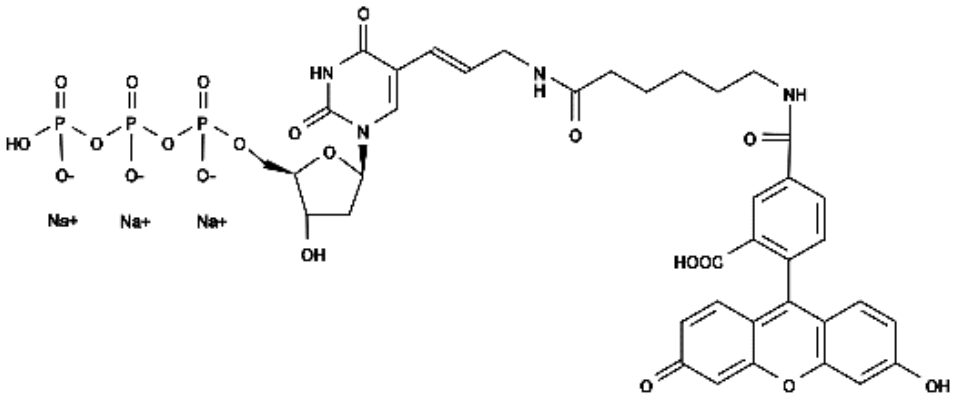
1. Identification

Product name **T7 Fluorescein RNA Labeling Kit**
RNA Synthesis with Modified Nucleotides
20 reactions

Cat. No. **RS028**

2. Description

The fluorescein labeled analog of uridine triphosphate **(Fluorescein-12-UTP)** is used for labeling RNA probes. Fluorescein-12-UTP is efficiently incorporated into RNA as substitute for its natural counterpart UTP via in vitro transcription with RNA polymerases and optimized reaction buffer. fluorescein-modified RNA probe may be detected directly via fluorescence spectroscopy.



T7 Fluorescein RNA Labeling Kit is designed to generate randomly fluorescein-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
Fluorescein-12-UTP (5mM)	10 µl
DTT (100 mM)	100 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 Fluorescein RNA Labeling Kit components are stable up to the expiry date specified with the product.

5. Applications

Fluorescein-labeled RNA is used for hybridization to

- in situ hybridization to tissues
- Southern blots
- plaque or colony lifts or
- RNase protection experiments

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 fluorescein-labeled RNA.
- The recommended molar ratio of Fluorescein-12-UTP to standard NTP is 1:3 or 1:2.

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	5 µl	2.5 mM
5. 10 mM UTP	0.4 µl	0.2 mM
6. 5mM Fluorescein-12-UTP	0.4 µl	0.1 mM
7. Template DNA	X µl	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.

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Incorporation rate of fluorophore:

The efficiency of RNA labeling can be estimated by calculating the ratio of incorporated fluorophores to the number of bases (dye / base).
[Please note: Blanc correction with probe buffer solution is required.]

1. Measurement of the nucleic acid-dye conjugate absorbance:
Measure the absorbance of the labeled RNA fragment at 260 nm (A_{260}) and at the excitation maximum (λ_{exc}) of dye (A_{dye}).
2. Correction of A260 reading:
To obtain an accurate nucleic acid absorbance measurement, the contribution of the dye at 260 nm needs to be corrected. Use the following equation:
$A_{base} = A_{260} - (A_{dye} \times CF_{260})$
Correction Factor for Fluorescein: $CF_{260} = 0.32$
3. Calculation of dye to base ratio by the law of Lambert-Beer ($A = c \times \varepsilon \times d$):

dye/base ratio = $(A_{dye} \times \varepsilon_{base}) / (A_{base} \times \varepsilon_{dye})$
Extinction coefficients:
Fluorescein: $\varepsilon_{dye} = 83{,}000\ cm^{-1}\ M^{-1}$
ssRNA: $\varepsilon_{base} = 12{,}030\ cm^{-1}\ M^{-1}$ (average, 50% GC)

4. Calculation of the degree of labeling (DOL)
The degree of labeling (DOL) indicates the number of dyes per 100 bases.
DOL = 100 x dye/base ratio

Example: A dye/base ratio of 0.02 corresponds to a DOL of 2 that corresponds to 2 dyes per 100 bases.