

Atto 647N RNA Labeling Kit Fluorescent labeling of RNA probes

Cat. No.	Amount
FP-220-647N	10 reactions

For *in vitro* use only!
Quality guaranteed for 12 months
Store at -20°C, avoid frequent thawing and freezing
Protect from light - work under low light conditions!

T7 RNA Polymerase (red cap)
2.5 µl 200 U/µl
T7 Reaction Buffer (green cap)
40 µl 5x conc.
Atto 647N Labeling Mix (purple cap)
40 µl 5x conc.
T7 Control Template (white cap)
2 µg 1423 bp (including T7 promoter sequence)
RNase Inhibitor (yellow cap)
5 µl 40 U/µl RNase inhibitor in storage buffer with
50% glycerol (v/v)
RNase-Free Water (white cap)
1.2 ml
RNase-Free DNase (yellow cap)
10 µl 1 U/µl
EDTA (yellow cap)
10 µl 0.5 M (pH 8.0)

Introduction

This kit is designed for 10 one-step RNA labeling reactions of 20 µl each.

Atto 647N UTP is efficiently incorporated into RNA by *in vitro* transcription using T7 RNA polymerase. Due to the excellent stability and quantum yield of the Atto 647N fluorophore, the resulting fluorescent RNA probe is perfectly suited for fluorescence *in situ* hybridization (FISH), Northern and Southern Blotting and nuclease protection assays (NPA).

Template DNA

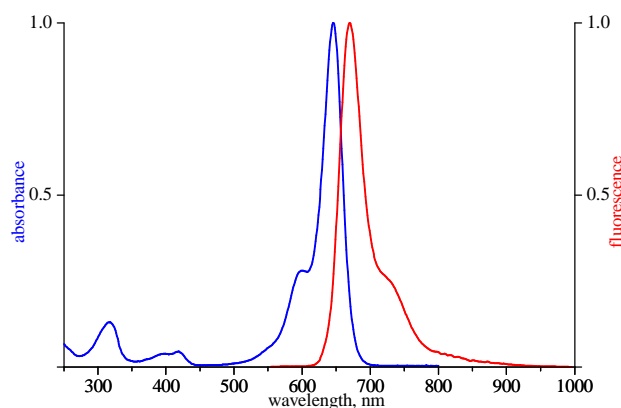
Any linearized DNA sequence containing a T7 promoter site can be used as template.

Minimum promoter sequence for efficient transcription:
5'-TAATACGACTCACTATAGGGAGA...-3'

└─ Start of transcription

Spectroscopic data of Atto 647N

Excitation maximum: $\lambda_{Ex} = 644 \text{ nm}$
Emission maximum: $\lambda_{Em} = 669 \text{ nm}$
Extinction coefficient: $\epsilon_{max} = 150,000 \text{ cm}^{-1} \text{ M}^{-1}$
Correction factor: $CF_{260 \text{ nm}} = 0.06$



Atto 647N excitation and emission spectra

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Experimental Protocol

The protocol is optimized for 0.3-1 µg linearized T7-template-DNA in a 20 µl reaction assay, typical yields range from 1.5-3 µg labeled RNA.

1. *In vitro* transcription

Add the following components on ice to a nuclease-free microtube:

Component	stock conc.	final conc.	1 assay
T7 Reaction Buffer	5x	1x	4 µl
Labeling Mix	5x	1x	4 µl
RNase Inhibitor ¹	40 U/µl	0.5-1 U/µl	0.25-0.5 µl
T7 RNA Polymerase	200 U/µl	2.5 U/µl	0.25 µl
linearized T7-template-DNA			0.3-1 µg
RNase-free water			Fill up to 20 µl

Mix gently by pipetting up and down and incubate (37°C, 30 min).

2. DNase treatment

Add RNase-free DNase (1 µl, 1 U/µl) to the reaction product and incubate (37°C, 15 min) to remove template DNA.

Add EDTA (1 µl, 0.5M) to stop the reaction.

3. Purification of the probe (optional)

Apply appropriate spin columns or LiCl precipitation to purify your labeled probe if necessary.

Please note that purification is an optional step and that RNA purification material is not provided with the kit!

4. Storage of the probe

The probe is stable at -80°C for up to two weeks. Protect from light!

¹ Addition of 10-20 U RNase inhibitor per 20 µl assay is recommended (and may be essential when working with low amounts of template DNA)

Background Information

Labeling Efficiency and RNA Yield

The labeling efficiency and RNA yield is strongly dependent on the DNA template and on the fluorophore that is incorporated.

Typical labeling degrees and RNA yields are given in the table below. Experiments were performed using 500 ng template DNA (523 bp λ-DNA fragment containing a T7 promoter site) in a 20 µl reaction assay.

nucleotide	Ex/Em	yield	DOL
UTP-ATTO 488	501/523	2 µg	16.5
UTP-ATTO 532	532/553	3 µg	3.3
UTP-ATTO 550	554/576	1.5 µg	2.9
UTP-ATTO 647N	644/669	2 µg	1.5
UTP-ATTO 680	680/700	2.5 µg	2.2

DNase treatment

Removal of template DNA by DNase treatment is recommended (especially for subsequent hybridization assays) otherwise loss of sensitivity may occur.

Purification of the probe

Probe purification is usually not necessary in hybridization assays. For some applications such as nuclease protection assays (NPA) however, it is essential to ensure absence of truncated probes and thus, purification of the transcript after the labelling reaction is recommended.

We recommend spin columns with silica-based membranes for purification, but LiCl precipitation is suitable, too.

For further information and related products, please visit us at: **www.jenabioscience.com**