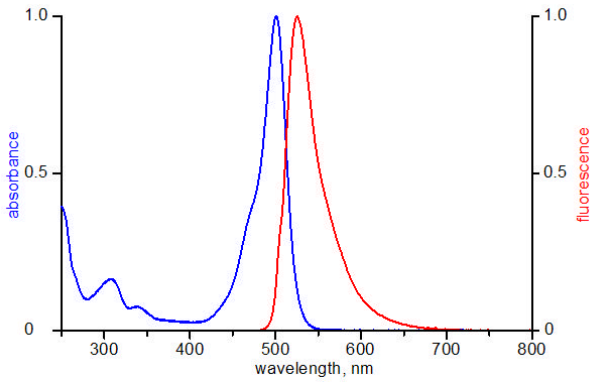
DATA SHEET

Jena Bioscience

HighYield T7 Atto488 RNA Labeling Kit

Preparation of randomly Atto488-modified RNA probes by *in vitro* transcription with UTP-ATTO-488

Cat. No.	Amount
RNT-101-488-S	20 reactions x 20 µl
RNT-101-488-L	50 reactions x 20 µl



Excitation and Emission spectrum of ATTO 488

For *in vitro* use only!

Shipping: shipped on gel packs

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Spectroscopic Properties: λ_{exc} 500 nm, λ_{em} 520 nm, ε 90.0 L mmol^{-1} cm^{-1} (Tris-HCl pH 7.5)

Description:
HighYield T7 Atto488 RNA Labeling Kit is designed to produce randomly Atto488-modified RNA probes via *in vitro* transcription. Such probes are ideally suited for *in situ* hybridization and Northern Blot experiments.
UTP-ATTO-488 is efficiently incorporated into RNA as substitute for its natural counterpart UTP using an optimized reaction buffer and T7 RNA Labeling Polymerase Mix. ATTO488 possesses, in contrast to Fluorescein, a excellent photostability.
35 % UTP-ATTO-488 substitution typically results in an optimal balance between reaction and labeling efficiency. Individual optimization of UTP-ATTO-488/UTP ratio however, can easily be achieved with the single nucleotide format.
The resulting Atto488-modified RNA probe can subsequently be detected by fluorescence spectroscopy.
The kit contains sufficient reagents for 20 labeling reactions of 20 µl each (35 % UTP-ATTO-488 substitution, 2.5 mM ATP, GTP, CTP, 0.2 mM UTP, 0.1 mM UTP-ATTO-488).

Content:

HighYield T7 RNA Labeling Polymerase Mix
RNT-101-488-S: 2x 40 µl, incl. RNase inhibitor and 50 % glycerol (v/v)
RNT-101-488-L: 3x 40 µl, incl. RNase inhibitor and 50 % glycerol (v/v)

HighYield T7 Reaction Buffer
1x 200 µl (10x), HEPES-based

ATP - Solution
1x 100 µl (100 mM)

GTP - Solution
1x 100 µl (100 mM)

CTP - Solution
1x 100 µl (100 mM)

UTP - Solution
1x 100 µl (100 mM)

UTP-ATTO-488
RNT-101-488-S: 2x 20 µl (1 mM)
RNT-101-488-L: 5x 20 µl (1 mM)

T7 G-initiating control template (1.4 kbp)
1x 10 µl (200 ng/µl), 1.4 kbp PCR fragment plus T7 class III phi6.5 promotor resulting in approx. 1400 nt RNA transcript

PCR-grade water
1x 1.2 ml

DTT
1x 100 µl (100 mM)

HighYield T7 Atto488 RNA Labeling Kit

Preparation of randomly Atto488-modified RNA probes by *in vitro* transcription with UTP-ATTO-488

To be provided by user
T7 Promotor-containing DNA template
RNA purification tools
RNAse-free DNAse I (optional)

1. Important Notes (Read before starting)

1.1 Prevention of RNAse contamination

Although a potent RNase Inhibitor is included, creating a RNAse-free work environment and maintaining RNAse-free solutions is critical for performing successful in vitro transcription reactions. We therefore recommend

- to perform all reactions in sterile, RNAse-free tubes using sterile pipette tips.
- to wear gloves when handling samples containing RNA.
- to keep all components tightly sealed both during storage and reaction procedure.

1.2 Template requirements

- Template type: Linearized plasmid DNA or PCR products containing a double-stranded T7 class II phi2.5 or class III phi6.5 promotor region upstream of the target sequence. Transcription initiation from T7 class III promotor is generally more efficient than initiation from T7 class II promotor.
  Minimum T7 promotor sequences:

  T7 class III phi6.5 promotor
  5'-TAATACGACTCACTATAG*NN*...-3'
  Bold: First base incorporated into RNA, *NN*: ideally *CG*

  or

  T7 class II phi2.5 promotor
  5'-TAATACGACTCACTATTAG*NN*...-3'
  Bold: First base incorporated into RNA, *NN*: ideally *CG*

- Template quality: DNA template quality directly influences yield and quality of transcription reaction. Linearized plasmid DNA needs to be fully digested and to be free of contaminating RNase, protein and salts. We recommend selecting restriction enzymes that generate blunt ends or 5'-overhangs and purification by phenol/chloroform extraction. A PCR mixture can be used directly however, better yields will usually be obtained with purified PCR products (e.g. via silica-membrane based purification columns).

2. Preparation of working solutions

2.1 Preparation of 10 mM ATP/CTP/GTP working solution

- Thaw 100 mM ATP, 100 mM CTP and 100 mM GTP solutions on ice, voretex and spin-down briefly.
- Prepare a 1:10 dilution with PCR-grade 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 PCR-grade water).
- 10 mM ATP/CTP/GTP working solution can be stored at -20°C. Avoid freeze/thaw cycles.

2.2 Preparation of 10 mM UTP working solution

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

3. In vitro Transcription protocol

The protocol is optimized for 0.5 µg - 1 µg DNA template.
An optimal balance between reaction and labeling efficiency is typically achieved with 35% UTP-ATTO-488 substitution following the standard protocol below however, individual optimization might improve results for individual applications (e.g. variation of UTP-ATTO-488/UTP ratio).

- Place HighYield T7 RNA Labeling Polymerase Mix on ice.
- Thaw all remaining components at room temperature (RT), mix by voretexing and spin down briefly.
- Assemble all components at RT to a nuclease-free microtube (sterile pipette tips) in the following order:
- Mix PCR-grade water, HighYield T7 Reaction Buffer and DTT by voretexing and spin down briefly.
- Add nucleotide solutions and template DNA, vortex and spin down briefly.
- Add HighYield T7 RNA Labeling Polymerase Mix vortex and spin down briefly.
- Incubate for 30 min at 37 °C in the dark (e.g. PCR cycler). Depending on the RNA probe individual optimization may increase product yield (2h – 4h at 37 °C).

HighYield T7 Atto488 RNA Labeling Kit

Preparation of randomly Atto488-modified RNA probes by *in vitro* transcription with UTP-ATTO-488

Component	Volume	Final concenctration
PCR-grade water	X µl	
HighYield T7 Reaction Buffer (10x)	2 µl	1x
100 mM DTT	2 µl	
10 mM ATP/CTP/GTP working solution (s. 2.1)	5 µl	2.5 mM
10 mM UTP working solution (s. 2.2)	0.4 µl	0.2 mM
1 mM UTP-ATTO-488	2 µl	0.1 mM
Template DNA	X µl	0.5 - 1 µg
HighYield T7 RNA Labeling Polymerase Mix	2 µl	
Total volume	20 µl	

Please note: Reagents for the following steps are not provided within this kit.

DNA template removal (optional)
Depending on the down-stream application, removal of template DNA might be required. We recommend a salt-resistant, high efficiency DNAase such as Turbo™DNAse (ThermoFisher). Follow the manufacturer instructions.

RNA purification
Purification of RNA is required for certain applications such as measurement of Atto488-labelled RNA probe concentration. Spin column purification will remove proteins, salts and unincorporated nucleotides. Please follow the manufacturer instructions and ensure that the columns match with product size and possess a sufficient binding capacity (e.g. RNA Clean & Concentrator™ columns (Zymo Research) or Monarch® RNA Cleanup kit (NEB)). Other RNA purification methods such as LiCl precipitation may work but have not been tested.

Total RNA quantitation
RNA concentration can be determined by absorbance measurement at 260 nm (A_{260}) according to the Law-of-Lambert-Beer (A_{260} = 1 correspond to 40 µg/ml ssRNA).

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 A_{260} 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 Atto488: CF_{260} = 0.25

3. Calculation of dye to base ratio by the law of Lambert-Beer (A = c x ε x d):

dye/base ratio = $(A_{dye} \times \varepsilon_{base}) / (A_{base} \times \varepsilon_{dye})$

Extinction coefficients:
Atto488: ε_{dye} = 90,000 cm^{-1} M^{-1}
ssRNA: ε_{base} = 12,030 cm^{-1} M^{-1} (average, 50% GC)

3. 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.

Related Products:
Aminoallyl-UTP-ATTO-488, #NU-821-488