DATA SHEET

Jena Bioscience

HighYield T7 P&L RNA NMR Kit (2F-ATP)

Synthesis of 2-Fluoro-modified RNA

Cat. No.	Amount
RNT-203-S	15 reactions x 20 µl
RNT-203-L	50 reactions x 20 µl

For general laboratory use.

Shipping: shipped on gel packs

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months after date of delivery

Description:
HighYield T7 P&L RNA NMR Kit (2F-ATP) is designed to produce large amounts of 2-Fluoro-modified RNA via in vitro transcription with T7 RNA polymerase. The resulting internally modified (m)RNA can subsequently be used for structure determination *via* nuclear magnetic resonance (NMR) spectroscopy.

2F-ATP is incorporated instead of its natural counterpart in a non-perturbing way (intact base pairing properties). 2-Fluoro-modification simultaneously functions as a sensitive NMR reporter group (larger chemical shift dispersion than ^{1}H). The modified T7 RNA polymerase (T7 P&L RNA polymerase) with proline 266 replaced by leucine (P266L) has been associated with increased 5' homogeneity of transcripts especially when synthesized from A-initiating phi2.5 promoter.

The kit contains sufficient reagents for **15 reactions (S-Pack) or 50 reactions (L-Pack) á 20 µl (7.5 mM GTP, 7.5 mM 2-Fluoro-ATP, 7.5 mM UTP, 7.5 mM CTP)**. An individual optimization of 2-Fluoro-ATP concentration can easily be achieved with the single nucleotide format.
A 20 µl reaction yields about **80 µg RNA after 30 min incubation (1 µg T7 control template, 1.4 kb RNA transcript)**. Yields may however vary depending on the template (promotor design, sequence length, secondary structure formation).

Content:
HighYield T7 P&L RNA Polymerase Mix
RNT-203-S: 2x 40 µl incl. RNase inhibitor and 50 % glycerol (v/v)
RNT-203-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)

2-Fluoro-ATP
RNT-203-S: 3x 10 µl (100 mM)
RNT-203-L: 8x 10 µl (100 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 1400 nt RNA transcript

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PCR-grade water
1x 1.2 ml

DTT
1x 100 µl (100 mM)

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

Important Notes (Read before starting)

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.

Template requirements

- **Template type**: Linearized plasmid DNA or PCR products containing a double-stranded G-initiating T7 class III phi6.5 promotor region upstream of the target sequence.

  Minimum T7 promotor sequences:

  T7 class III phi6.5 promotor (G-initiating)
  5'-TAATACGACTCACTATA**G***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).

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***In vitro* Transcription protocol**

The general protocol is set up for 0.5 µg - 1 µg DNA template (refer to section 1.2 regarding template requirements), a final NTP concentration of 7.5 mM and 100% substitution of ATP by 2-Fluoro-ATP, respectively.

Depending on the RNA sequence and final application, individual reaction optimization may improve product yield and biological function (e.g. variation 2-Fluoro-ATP/ATP ratio , variation of template amount, variation of incubation time).

Component	Volume	Final conc.
PCR-grade water	X µl	
HighYield T7 Reaction Buffer (10x)	2 µl	1x
DTT (100 mM)	2 µl	10 mM
GTP (100 mM)	1.5 µl	7.5 mM
2-Fluoro-ATP (100 mM)	1.5 µl	7.5 mM
CTP (100 mM)	1.5 µl	7.5 mM
UTP (100 mM)	1.5 µl	7.5 mM
Template DNA	X µl	1 µg
HighYield T7 P&L RNA Polymerase Mix	2 µl	
Total volume	**20 µl**	

- Place HighYield T7 P&L RNA 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 P&L RNA Polymerase Mix vortex and spin down briefly.
- Incubate for 2h at 37°C in the dark (e.g. PCR cycler). Individual optimization may increase product yield (0.5h–4h at 37°C).

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

DNA template removal
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 2-Fluoro-labeled 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 corresponds to 40 µg/ml ssRNA).

Related Products:
2-Fluoro-ATP, #NU-145