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Apt-Get 2'-F T7 Transcription Kit

FOR SYNTHESIS OF NUCLEASE-RESISTANT 2'-FLUORO-PYRIMIDINE RNAs

T7 RNA Polymerase

– modified, optimized –
(Bacteriophage T7 of E. coli)

Cat. No.	Size
E0905-01	25 x 25 µl reactions
E0905-02	50 x 25 µl reactions

Unit Definition:
One unit is the amount of enzyme required to incorporate 1 nmol of labeled UTP into acid-soluble material in 1 hr at 37°C.

Storage Conditions:
Store at -20°C

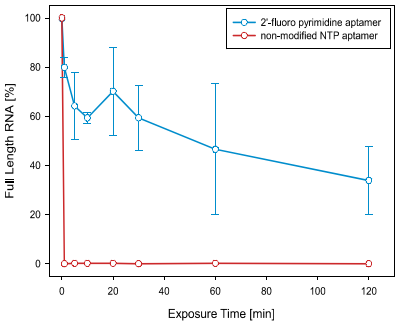

Figure 1: Determining stability, enhanced nuclease resistance and prolonged half-life of 2'F-pyrimidine RNA aptamers within nuclease-rich animal sera. 2'F-Py RNA aptamers (blue) and non-modified RNA aptamers (red) were incubated in cell culture medium containing fetal bovine serum, followed by recovery of nucleic acids. Radiolabelled full length aptamers were separated by non-denaturing PAGE. Band intensities of full length aptamers were visualized and quantified by autoradiography (8). Half-lifes of 2'-F Py aptamers are strongly depended on individual sequence and secondary structure features, and thus may vary in a broad range. For certain 2'F-Py aptamers, half lifes were reported to exceed 24 hour time-frames.

T7 transcription kit for synthesis of nuclease-resistant RNA. Contains a NTP mix with 2'-fluoro CTP and 2'-fluoro UTP, respectively. The kit features a modified T7 RNA polymerase with specific optimizations towards incorporation of 2'-fluoro modified nucleotides.

Description:

- Incorporation of 2'-fluoro-pyrimidines protects oligonucleotides from digestion by extracellular nucleases and prolongs oligonucleotide half-life within nuclease-rich environments (4).
- Reaction efficiencies of T7 assays strongly depend on balanced adjustment and high quality of all reaction components. Therefor, all kit components are carefully fine-tuned and specifically optimized for high efficient in vitro synthesis of modified RNA.
- 2'-fluoro pyrimidine RNAs are frequently applied in SELEX for aptamer synthesis as well as for development of nuclease-resistant small interfering RNAs (siRNA) with silencing ability (5).
- Synthesis of RNA with 2'-fluoro modified pyrimidines is the most common approach towards synthesis of nuclease-resistant fragments such as RNA aptamers (5), offering a good balance between enzyme performance and resistance of synthesized RNA towards nuclease digestion.
- 2'-fluoro modifications offer better secondary structure formation capabilities as compared to 2'-amino modifications, and display higher compatibility with downstream enzymatic manipulations as compared to 2'-O-methyl modified RNA.
- Half-life of 2'- fluoro modified aptamers depends on their precise sequence and secondary structure features, as well as on the composition and the nuclease load of the application environment. Typical half-life values in human or animal sera range between 30 minutes to several hours. In contrast, entirely non-modified RNAs are quickly degraded under these conditions and half lifes are usually too short for being reliably measured.
- 2'-fluoro pyrimidine RNA is compatible to reverse transcription with NG dART Reverse Transcriptase (Cat. No. 0801).
- NTP mix consists of 2'-deoxy-2'-fluorocytidine 5'-triphosphate, 2'-deoxy-2'-fluorouridine 5'-triphosphate as well as non-modified ATP and GTP, respectively. The NTP mix does not contain any non-modified CTP or UTP, respectively.

Quality Control:

All preparations are assayed for contaminating exonuclease, endonuclease , for nonspecific RNase and single- and double-stranded DNase activities. Typical preparations are greater than 90 % pure, as judged by SDS polyacrylamide gel electrophoresis.

References:

1. Chamberlin, M. and Ring, J. (1973) J. Biol. Chem. 248, 2235-2244.
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3. Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, second edition, pp. 10.27-10.37, Cold Spring Harbour Laboratory, Cold Spring Harbour.
4. Pieken W.A. *et al.* (1991) Science 19 253 (5017) 314-317.
5. Hernandez F.J. *et al.* (2012) Nucleic Acid Ther. 22 (1) 58-68.
6. Aurup H. *et al.* (1992) Biochemistry 13, 31 (40) 9636-9641
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8. Meyer C., Berg K. *et al.* (2014) RNA Biology 11 (1), 1-9

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Apt-Get 2'-F T7 Transcription Kit

RNA TRANSCRIPTION PROTOCOL

Kit – Package Contents:

Kit component:	25 rxn	50 rxn
5 x T7 Reaction Buffer	150 µl	300 µl
2'-F NTPs mix, 25 mM each*	37.5 µl	75 µl
Apt-Get 2'-F T7 RNA Polymerase	12.5 µl	25 µl
RNase-free water	0.5 ml	1.0 ml

* Mix consists of non-modified ATP and GTP, and of 2'-F CTP and 2'-F UTP, respectively.

Reaction Setup:

Perform reaction assembly on ice by mixing the following components.

5 x reaction buffer	5 µl
2'-F Py NTP mix 25 mM each	1.5 µl
DNA template*	1-2 µg
Apt-Get 2'F-T7 RNA Polymerase**	0.5 µl
RNase-free water	@ 25 µl
TOTAL	**25 µl**

Incubate 1- 2 hours at 37°C or to max. 42°C.

Check RNA transcription by denaturing polyacrylamide gel electrophoresis (PAGE).

Remarks:

* High purity of the DNA template is important for obtaining maximum reaction yield. If run off transcription is applied, make sure to avoid RNase A contamination due to insufficient plasmid preparation. We recommend using our RNase free Plasmid DNA Purification kit (Cat. No. E3500), which works excellent for preparing RNase free plasmid DNA. In case, T7 template DNA is a PCR fragment, remove primers (recommended: PCR/DNA Clean-Up Purification Kit Cat.No. E3520). Confirm DNA homogeneity on an agarose gel.

** 0.2 µl of T7 RNA polymerase is most efficient for labeling, higher enzyme amounts are recommended for work on preparative scale.

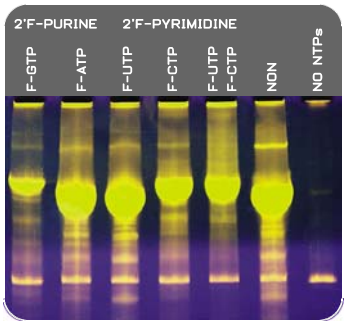

Figure 2: T7 transcription with EURx Apt-Get 2'-F T7 RNA Polymerase for incorporation of 2'-F modified NTPs. In each lane, the denoted 2'-F NTP was used in spite of its non-modified counterpart. NON; Reaction with all four standard NTPs. Transcript length: 500 nt RNA. 10 µl of each transcription assay was loaded on a 7% [w/v] polyacrylamide gel with 8 M urea.

Note: Non-modified, wild type T7 RNA polymerase is not capable of using 2'-F NTP analogues as substrate.

Sequence Data for PCR Primer Design:

T7 promotor sequence (required):

```
   |   Recognition   |     > Transcription
   |-17            -5|   -1|
5'- T AAT ACG ACT CAC TAT A -3'
```

Example for a frequently used, known-to-work T7 PCR primer (5):

5'-GAA ATT AAT ACG ACT CAC TAT AGG - sequence specific primer part -3'

T7 RNA polymerase synthesizes from 5'- in 3'-direction and therefore requires template orientation from 3'- to 5'- direction. The complementary strand to the T7 promotor (i.e. to the primer sequence) serves as template.

5'- GAAA**TTAATACGACTCACTATA**GG -3' non-template

3'- CTTT**AATTATGCTGAGTGATAT**CC -5' template

Generic T7 terminator sequences (optional):

1) Class I terminator, hairpin with GC-rich stretch followed by T-rich element (4)

5'-AGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTIGCT GAAAG-3'

2) Class II terminators / pausing occurs 7-8 nt downstream the ATCTGTT motif, termination occurs at the underlined positions (4).

5'-ATCTGTTacagtctcct-3' or

5'-ATCTGTTttcttgcaag-3'

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