Apt-Get
2'-F T7 Transcription Kit
2'-Fluoro-Pyrimidine Set

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

T7 transcription kit for synthesis of nuclease-resistant RNA. Contains a mix of NTPs with 2'-fluoro CTP and 2'-fluoro UTP. The T7 RNA Polymerase is optimized towards incorporation of 2'-fluoro modified nucleotides.

Storage Conditions:

Store at -20°C.

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. *Pieken W.A. et al. (1991) Science 19 253 (5017) 314-317.*
2. *Hernandez F.J. et al. (2012) Nucleic Acid Ther. 22 (1) 58–68.*
3. *Aurup H. et al. (1992) Biochemistry 13, 31 (40) 9636-9641.*
4. *Sousa R., Padilla R. (1995) EMBO J. 15 14 (18) 4609-2461.*
5. *Meyer C., Berg K. et al. (2014) RNA Biology 11 (1), 1–9.*

Description:

- Incorporation of 2'-fluoro-pyrimidines protects oligonucleotides from digestion by extracellular nucleases and prolongs oligonucleotide half-life in the nuclease-rich environment (1, 3).
- The efficiency of T7 assays strongly depends on balanced adjustment and high quality of all reaction components. The kit incorporates carefully optimized reagents and conditions that improves the efficiency of RNA synthesis.
- 2'-fluoro pyrimidine RNAs are frequently applied in SELEX for aptamers synthesis as well as for development of nuclease-resistant small interfering RNAs (siRNA) with silencing ability (2).
- 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, 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-lives are usually too short for being reliably measured.
- 2'-fluoro pyrimidine RNA is compatible with reverse transcription reaction with NG dART Reverse Transcriptase (Cat. No. E0801).
- 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 NTPs mix does not contain any non-modified CTP or UTP.

Kit Components:

COMPONENT:	E0905-01	E0905-02
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 ml

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

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

In vitro T7 transcription assay, 25 µl:

1. Mix:

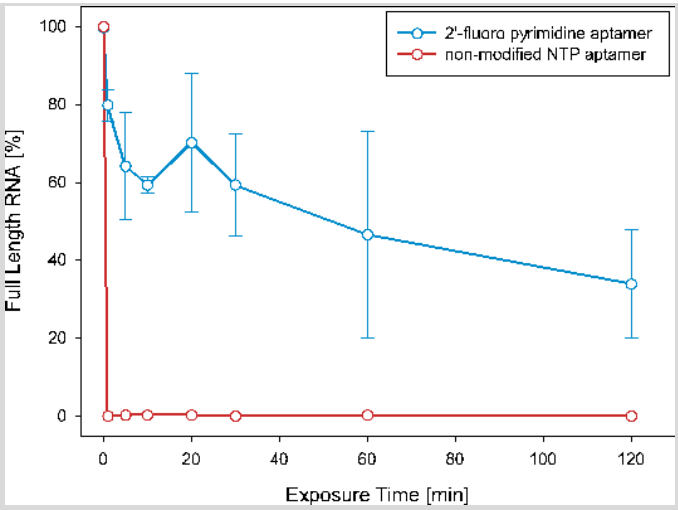
Component:	Amount:
5 x T7 Reaction Buffer	5 µl
2'-F NTPs mix, 25 mM each	1.5 µl
DNA template*	1-2 µg
Apt-Get 2'-F T7 RNA Polymerase**	0.5 µl
RNase-free Water	to 25 µl

2. Incubate 1-2 hours at 37°C, then check transcription on appropriate denaturing polyacrylamide gel.

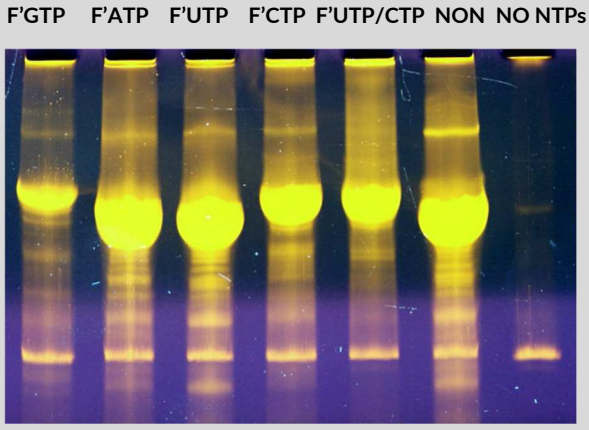
NOTES:

*High purity of the 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: EURx 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.



Determining stability, enhanced nuclease resistance and prolonged half-life of 2'-F pyrimidine 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. Radiolabelled full length aptamers were separated by non-denaturing PAGE. Band intensities of full length aptamers were visualized and quantified by autoradiography (5). 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 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 is 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: Wild type T7 RNA Polymerase is not able to use as substrate any 2'-F NTP analoques (4).

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