DATA SHEET

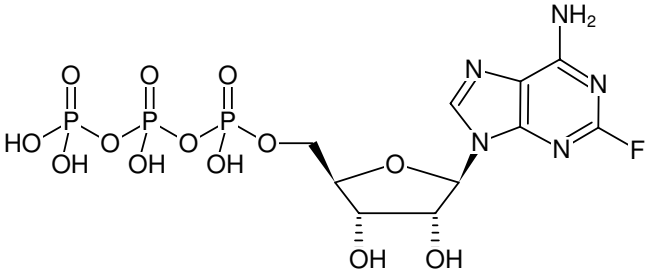
Jena Bioscience

2-Fluoro-ATP

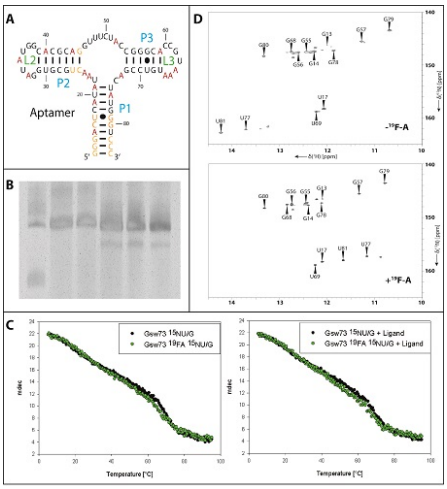
(2F-ATP)

2-Fluoro-adenosine-5'-triphosphate, Sodium salt

Cat. No.	Amount
NU-145S	10 µl (100 mM)
NU-145L	5 x 10 µl (100 mM)



Structural formula of 2-Fluoro-ATP



For general laboratory use.

Shipping: shipped on gel packs

Storage Conditions: store at -20 °C

Short term exposure (up to 1 week cumulative) to ambient temperature possible.

Shelf Life: 12 months after date of delivery

Molecular Formula: $C_{10}H_{15}N_5O_{13}P_3F$ (free acid)

Molecular Weight: 525.17 g/mol (free acid)

Exact Mass: 524.99 g/mol (free acid)

CAS#: 1492-62-2 (free acid)

Purity: ≥ 95 % (HPLC)

Form: solution in water

Color: colorless to slightly yellow

Concentration: 100 mM - 110 mM

pH: 7.5 ±0.5

Spectroscopic Properties: λ_{abs} 261 nm, ε 14.3 L mmol^{-1} cm^{-1} (Tris-HCl pH 7.5)

Description:
Figure 1A: Schematic representation of the secondary structure of the Gsw^{apt}. The stems are named in blue and the loops are named in green. All fluorinated bases are labeled in red.
Figure 1B: 10% PAA-Gel of the transcription samples containing from left to right: low range ssRNA-marker, transcription with unlabeled dNTPs, ^{15}N-UTP, ^{15}N-GTP, 2F-ATP, ^{15}N-UTP-GTP + 2F-ATP.
Figure 1C: CD-melting curve of the Gsw^{apt} in the ligand bound state. The black curve represents the unlabeled RNA and the green curve represents the ^{19}F-labeled RNA. The melting curves were recorded in the absence (left) and presence (right) of the ligand.
Figure 1D: ^{1}H-^{15}N-HSQC of the Gsw^{apt}. The spectrum of the unlabeled RNA is shown on the top and the spectrum of the ^{19}F-labeled RNA is shown at the bottom.

RNA Structure Determination by NMR with 2F-ATP

Application Note

By courtesy of

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Multidimensional NMR studies of RNAs are based on the correlation between magnetic properties of protons and of other NMR-active atoms. In nucleic acids these atoms are usually ^{13}C, ^{15}N and ^{31}P. Whereas ^{31}P is at 100% natural abundance, the other NMR-active, non-radioactive isotopes have low natural abundance. The methodic challenges are to artificially enrich or introduce NMR active isotopes of ^{13}C and ^{15}N into the molecules without affecting their overall structural integrity. Besides the atoms present in RNA, also introduction of different NMR reporter signals is of considerable interest in NMR studies of large RNAs.
In this application note we substituted the hydrogen at positions 2 in the base of adenosine (Ade-2H) with ^{19}F, which has a comparable gyromagnetic ratio as ^{1}H but exhibits larger chemical shift dispersion. The Ade-2H position was chosen as the attached nucleus is spatially close enough to the base pairing but does not show J-coupling to other protons.

We introduce ^{19}F labelling for the 73 nucleotides comprising RNA aptamer region of the guanine sensing riboswitch from *Bacillus subtilis* (Gsw^{apt}, shown in Fig 1A). Due to the inherent low sensitivity, samples of RNA for NMR spectroscopic studies require high concentration of the solute, in this case of the ^{19}F labeled RNA. Therefore it was synthesized in large scale (0.15 mg) by *in vitro* transcription with 2F-ATP complementing ATP. The composition of the transcription mix is shown in Table 1. The concentrations of $Mg(OAc)_2$, DNA-template, nucleotides and YIPP were optimized for highest transcription efficiency. Several in vitro transcriptions with different nucleotide composition were performed overnight at 37 °C (Figure 1B). The yield of ^{19}F labeled Gsw^{apt} synthesized within a transcription reaction containing also ^{15}N-GTP and ^{15}N-UTP was compared with the yield in a transcription reaction containing solely ^{15}N-GTP and ^{15}N-UTP as isotope labeled compounds. All NTP compositions led to a single 73 nt RNA product. The transcription efficiency, measured by HPLC, is 1.9% (±0.4%) for all transcriptions including 2F-ATP compared to 2.1% (±0.5%) for the transcriptions with unlabeled or ^{15}N-labeled nucleotides only.

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Table 1: Transcription parameters

Fixed parameters	
Tris HCl pH 8.1	40 mM
DTT	4 mM
Spermidine	1 mM
BSA	50 µg/ml
Triton X-100	0.01 %
Polyethylenglycol	80 mg/ml
T7 RNA-Polymerase	70 µg/ml
Variable parameters	
$Mg(OAc)_2$	27.25 mM
DNA template	67 nM
ATP/2F-ATP	2 mM
CTP	2.24 mM
GTP	2.36 mM
UTP	2 mM
YIPP	0.002 U/µl

RNA stability was investigated by CD-spectroscopy (Figure 1C). In denaturation experiments, the melting curves of the ^{19}F-labeled and the unlabeled RNA show an identical progression. Without ligand, the ΔTm = Gsw^{apt} - ^{19}F-Gsw^{apt} = 4 K. In the ligand bound state, the ΔTm = 2 K. Furthermore, ^{1}H-^{15}N-HSQCs were recorded with the ^{19}F-labeled and the unlabeled RNA (Fig.2D). The signals corresponding to protons of G-C and G-U-base pairing are identical for both RNAs. In contrast, the signals of the A-U base pairing protons display a highfield shift of $<\Delta\delta^{1}H> = -2.6$ ppm.

Related Products:
HighYield T7 P&L RNA NMR Kit (2F-ATP), #RNT-203

Selected References:
Sochor *et al.* (2016) S(19)F-labeling of the adenine H2-site to study large RNAs by NMR spectroscopy. *J. Biomol. NMR* **64 (1)**:63.

Stockman (2008) 2-Fluoro-ATP as a versatile tool for 19F NMR-based activity screening. *J. Am. Chem. Soc.* **130 (18)**:5870.

Scott *et al.* (2004) Enzymatic synthesis and 19F NMR studies of 2-fluoroadenine-substituted RNA. *J. Am. Chem. Soc.* **126 (38)**:11776.