Monolith Protocol MO-P-022

DNA Aptamer – AMP (C6 amino)

The DNA aptamer for adenosine is a highly conserved sequence that is a widely used model aptamer for biosensor development. It also binds ADP and ATP, and with slightly weaker affinity AMP. Aptamer with an NH_2-modification at the 5' end can be fluorescently labeled using an amino-reactive NHS-dye.

DNA – small molecule interaction | aptamer

A1. Target/Fluorescent Molecule

AMP aptamer

A2. Molecule Class/Organism

DNA aptamer

A3. Sequence/Formula

5' C6-NH_2 ACC TGG GGG AGT ATT GCG GAG GAA GGT 3'

A4. Purification Strategy/Source

metabion international AG

A5. Stock Concentration/Stock Buffer

0.87 mg/mL | 100 µM
ddH_2O

A6. Molecular Weight/Extinction Coefficient

8664 Da
273,300 $M^{-1}cm^{-1}$ (ε_{260})

A7. Dilution Buffer

20 mM Tris-HCl, pH 7.8, 300 mM NaCl, 5 mM $MgCl_2$, 0.05% TWEEN® 20

A8. Labeling Strategy

Monolith Protein Labeling Kit RED – NHS 2nd Generation (MO-L011, NanoTemper Technologies GmbH)
1* Labeling Buffer NHS | 1* Dye RED-NHS 2nd Generation (10 µg) | 1* B-Column

A9. Labeling Procedure

1. Add 35 µL of Labeling Buffer NHS to 10 µL of 100 µM AMP aptamer and mix well.
2. Add 5 µL of DMSO to Dye RED-NHS 2nd Generation (10 µg) to obtain a ~3 mM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
3. Add the 45 µL of the AMP aptamer solution from step 1 to the dissolved dye from step 2 to obtain 50 µL of a 20 µM DNA aptamer, 300 µM dye solution (15x protein concentration, 10% DMSO).
4. Incubate for 1 hour at room temperature in the dark.
5. In the meantime, remove the top cap of the B-Column and pour off the storage solution. Remove the bottom cap and place with adapter in a 15 mL tube.
6. Fill the column with dilution buffer and allow it to enter the packed resin bed completely by gravity flow. Discard the flow through collected. Repeat this step 3 more times.
7. Add 50 µL of the labeling reaction from step 3 to the center of the column and let sample enter the bed completely.
8. Add 500 µL of dilution buffer after the sample has entered and discard the flow through.
9. Place column in a new collection tube, add 500 µL of dilution buffer and collect the eluate.
10. Keep the labeled aptamer (~2 µM) on ice in the dark.

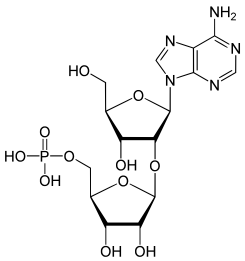
A10. Labeling Efficiency

Measurement of DNA concentration and degree of labeling (DOL) using a NanoDrop™:
nanotempertech.com/dol-calculator

Absorbance A_{260}	0.56	Protein concentration	2.0 µM
Absorbance A_{650}	0.12	Degree-of-labeling (DOL)	0.31

B1. Ligand/Non-Fluorescent Binding Partner

Adenosine monophosphate (AMP)



B2. Molecule Class/Organism

Nucleotide monophosphate

B3. Sequence/Formula

$C_{10}H_{14}N_5O_7P$

B4. Purification Strategy/Source

Sigma-Aldrich GmbH
01930

B5. Stock Concentration/Stock Buffer

17.4 mg/mL | 50 mM
20 mM Tris-HCl, pH 7.8, 300 mM NaCl, 5 mM $MgCl_2$, 0.05% TWEEN® 20

B6. Molecular Weight/Extinction Coefficient

347.22 Da

B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 50 mM AMP solution into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
2. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **16** to get an equal volume of 10 µL for all samples.
3. Mix 20 µL of labeled AMP aptamer with 180 µL of dilution buffer to obtain 200 µL of ~200 nM AMP aptamer.
4. Add 10 µL of ~200 nM labeled AMP aptamer to each tube from **16** to **1** and mix by pipetting.
5. Incubate for 5 minutes at room temperature in the dark before loading capillaries.

D1. MST System/Capillaries

Monolith NT.115 Red (NanoTemper Technologies GmbH)
Capillaries Monolith NT.115 (MO-K022, NanoTemper Technologies GmbH)

D2. MST Software

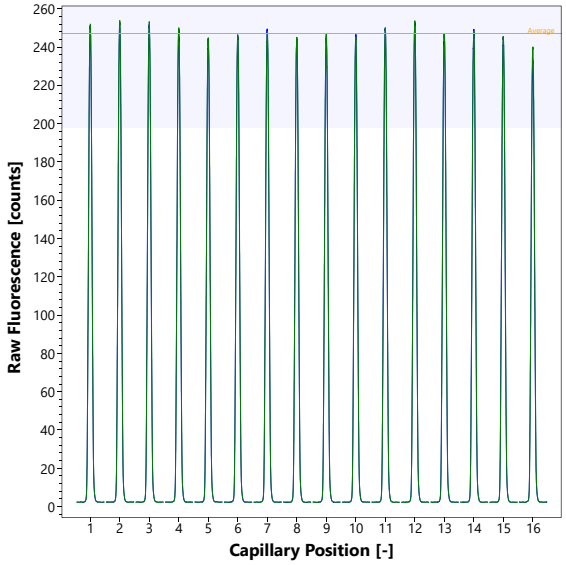
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

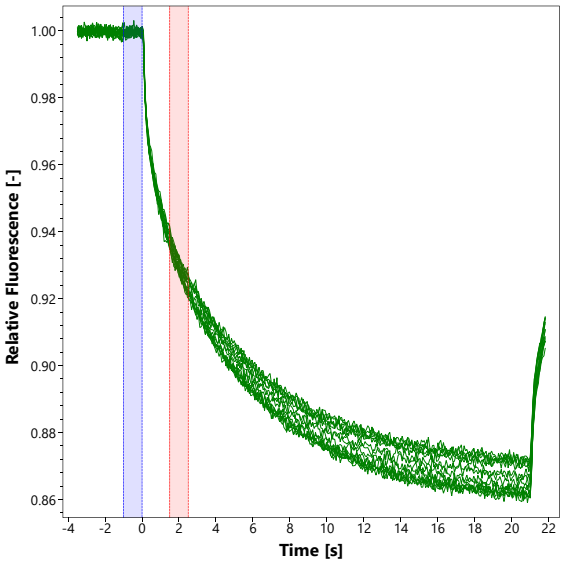
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

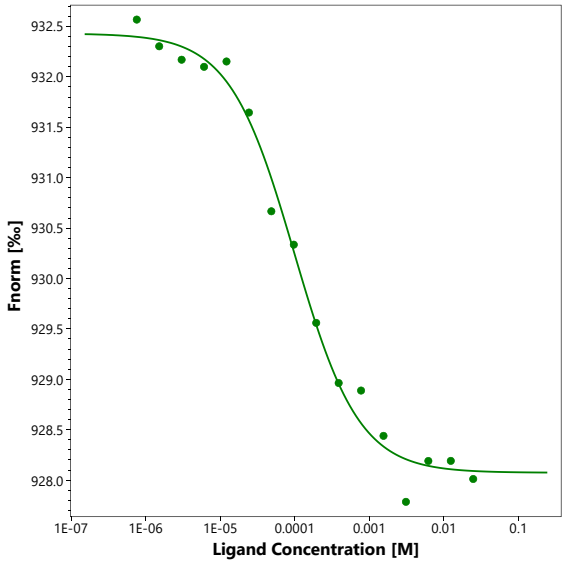
20 mM Tris-HCl, pH 7.8, 300 mM NaCl, 5 mM $MgCl_2$, 0.05% TWEEN® 20
100 nM DNA aptamer | 25 mM AMP – 763 nM | 25°C | low MST power | 20% excitation power

D4. MST Results (Capillary Scan/Time Traces/Dose Response)

K_d = 99.2 µM







D5. Reference Results/Supporting Results

K_d = 58 ± 2 µM Frontal chromatography analysis
Deng et al., Anal Chem 73 (2001) 5415-5421

E. Contributors

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