Monolith Protocol MO-P-021

DNA Aptamer – AMP

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.

DNA – small molecule interaction | aptamer

A1. Target/Fluorescent Molecule

AMP aptamer

A2. Molecule Class/Organism

DNA aptamer

A3. Sequence/Formula

5’ Cy5 ACC TGG GGG AGT ATT GCG GAG GAA GGT 3’

A4. Purification Strategy/Source

metabion international AG

A5. Stock Concentration/Stock Buffer

0.36 µg/ml | 40 nM
20 mM Tris-HCl, pH 7.8, 300 mM NaCl, 5 mM $MgCl_2$, 0.05% TWEEN® 20

A6. Molecular Weight/Extinction Coefficient

9019 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

5’ Cy5 labeled

A9. Labeling Procedure

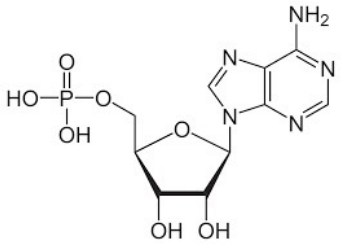
N/A

A10. Labeling Efficiency

HPLC-purified, 100% labeled DNA

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. Add 10 µL of 40 nM AMP aptamer to each tube from **16** to **1** and mix by pipetting.
4. 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

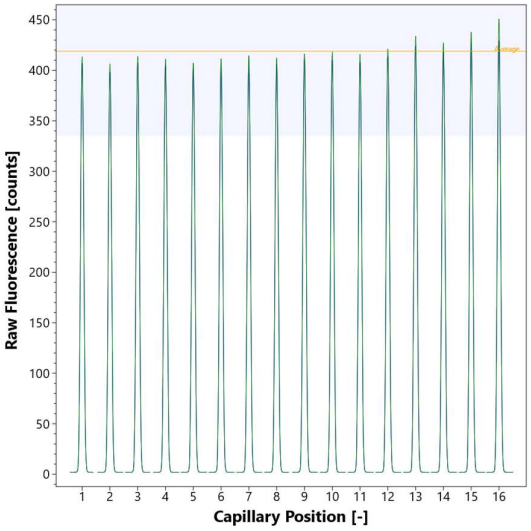
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

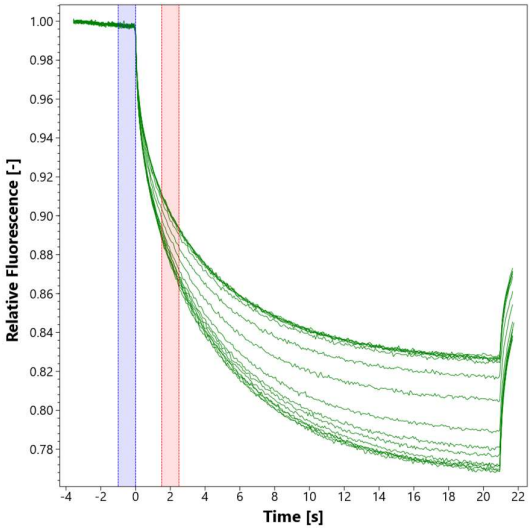
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

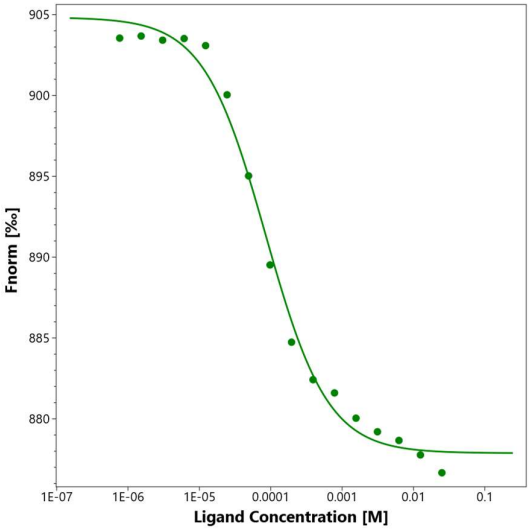
20 mM Tris, pH 7.8, 300 mM NaCl, 5 mM $MgCl_2$, 0.05% TWEEN® 20
20 nM DNA aptamer | 25 mM AMP – 763 nM | 25°C | low MST power | 20% excitation power

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

K_d = 85 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

Andreas Langer[1]

[1] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com