Monolith Protocol MO-P-072

Anti-Digoxigenin, Fab fragments – Digoxigenin-DNA

Anti-Digoxigenin, Fab fragments are Fab fragments from polyclonal anti-digoxigenin antibodies, which are used for the detection of digoxigenin-labeled compounds in ELISA or Western blot. Digoxigenin (DIG) is a steroid found exclusively in the flowers and leaves of the plants *Digitalis purpurea*, *Digitalis orientalis* and *Digitalis lanata* (foxgloves), where it is attached to sugars. It is widely used as nucleic acid label.

protein – small molecule interaction | Fab fragment

A1. Target/Fluorescent Molecule

Anti-Digoxigenin, Fab fragments

A2. Molecule Class/Organism

Fab fragment (from IgG1)
Sheep

A3. Sequence/Formula

N/A (polyclonal)

A4. Purification Strategy/Source

Roche, Sigma-Aldrich GmbH
1214667001

A5. Stock Concentration/Stock Buffer

1 mg
lyophilized

A6. Molecular Weight/Extinction Coefficient

~50 kDa
~70,000 $M^{-1}cm^{-1}$ (ε_{280}, determined with a NanoDrop® from a constituted 1 mg/mL stock solution)

A7. Dilution Buffer

Phosphate-buffered saline (PBS), pH 7.4, 0.01% Pluronic® F-127

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. Reconstitute 1 mg of Fab fragments in 1 mL of PBS to obtain a ~20 µM stock solution.
2. Transfer 100 µL of the 20 µM Fab stock solution into a fresh tube.
3. Add 25 µL of DMSO to Dye RED-NHS 2nd Generation (10 µg) to obtain a ~600 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
4. Mix 10 µL of the 600 µM dye solution with 90 µL of Labeling Buffer NHS to obtain 100 µL of a 60 µM dye solution (3x protein concentration).
5. Mix Fab and dye in a 1:1 volume ratio (200 µL final volume, 5% final DMSO concentration).
6. Incubate for 20 minutes at room temperature in the dark.
7. 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.
8. 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.
9. Add 200 µL of the labeling reaction from step 5 to the center of the column and let sample enter the resin bed completely.
10. Add 450 µL of dilution buffer after the sample has entered and discard the flow through.
11. Place column in a new collection tube, add 500 µL of dilution buffer and collect the eluate.
12. Keep the labeled Fab (~4 µM) on ice in the dark.

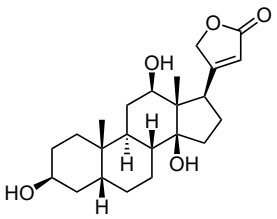
A10. Labeling Efficiency

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

Absorbance A_{205}	5.751	Protein concentration	~3.63 µM
Absorbance A_{650}	0.652	Degree-of-labeling (DOL)	~0.92

B1. Ligand/Non-Fluorescent Binding Partner

Digoxigenin-modified single-stranded DNA (DIG-DNA)



B2. Molecule Class/Organism

Steroid

B3. Sequence/Formula

5’ Digoxigenin TTT TTT 3’

B4. Purification Strategy/Source

Metabion GmbH

B5. Stock Concentration/Stock Buffer

100 µM
ddH_2O

B6. Molecular Weight/Extinction Coefficient

2,487 Da

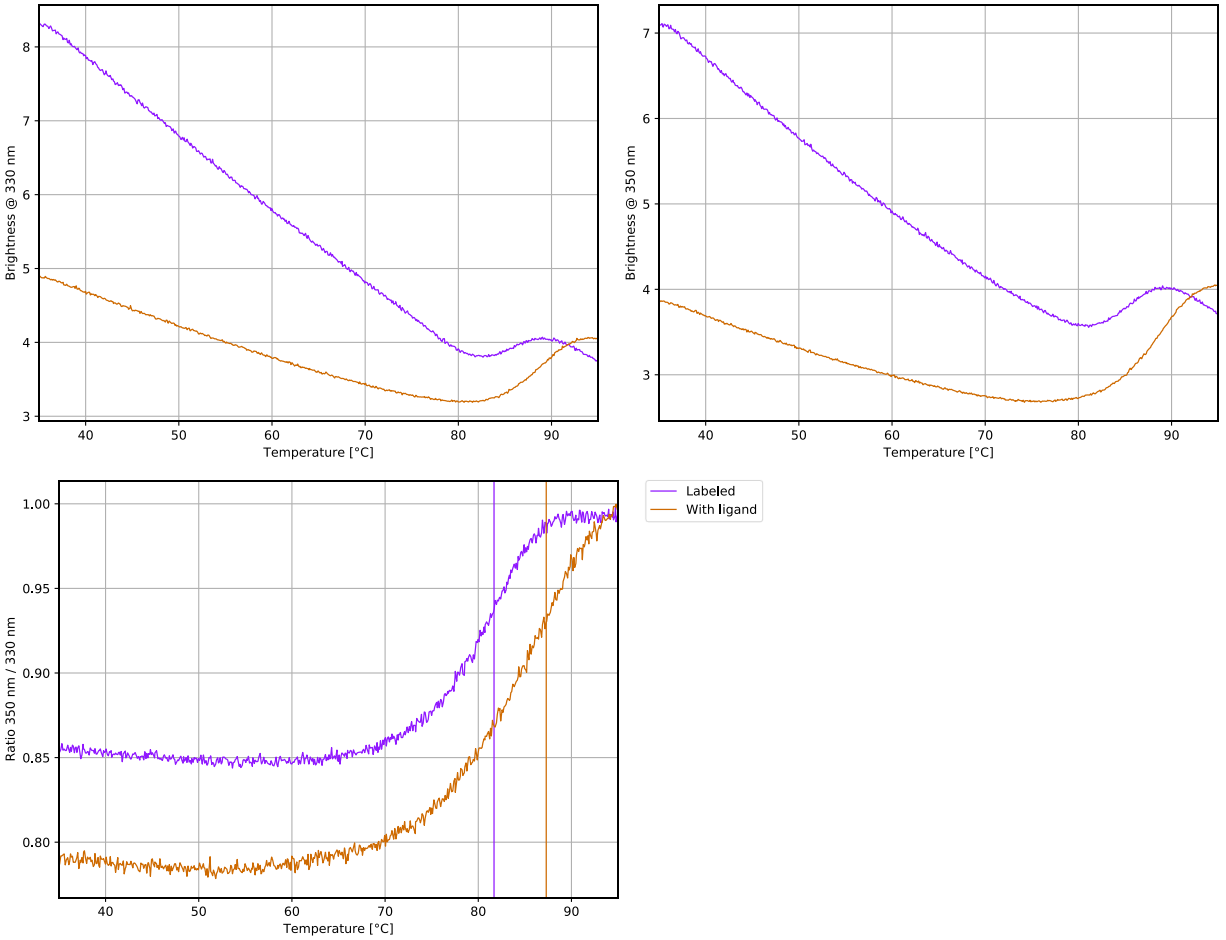
B7. Serial Dilution Preparation

1. Mix 2 µL of the 100 µM DIG-DNA stock with 98 µL of dilution buffer to obtain 100 µL of a 2 µM solution.
2. Prepare a PCR-rack with 16 PCR tubes. Mix 4 µL of the 2 µM DIG-DNA solution with 36 µL of dilution buffer in tube **1**. Then, transfer 20 µL of dilution buffer into tubes **2** to **16**.
3. Prepare a 1:1 serial dilution by transferring 20 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 20 µL from tube **16** to get an equal volume of 20 µL for all samples.
4. Mix 2 µL of labeled Fab (~4 µM) with 18 µL of dilution buffer to obtain 20 µL of a ~400 nM Fab solution.
5. Mix 2 µL of labeled Fab (~400 µM) with 398 µL of dilution buffer to obtain 400 µL of a ~2 nM Fab solution.
6. Add 20 µL of this solution to each tube from **16** to **1** and mix by pipetting.
7. Incubate tubes for 20 minutes at room temperature in the dark before loading capillaries.

C. Applied Quality Checks

Validation of structural integrity and functionality of labeled Fab fragments using Tycho NT.6:
nanotempertech.com/tycho

Labeled	3.5 µL of B-column eluate (~4 µM) + 7 µL of dilution buffer	T_i = 81.7°C
With ligand	3.5 µL of B-column eluate (~4 µM) + 7 µL of 2 µM DIG-DNA	T_i = 87.3°C



D1. MST System/Capillaries

Monolith NT.115^{PICO} Red (NanoTemper Technologies GmbH)
Capillaries Monolith NT.115 (MO-K022, NanoTemper Technologies GmbH)

D2. MST Software

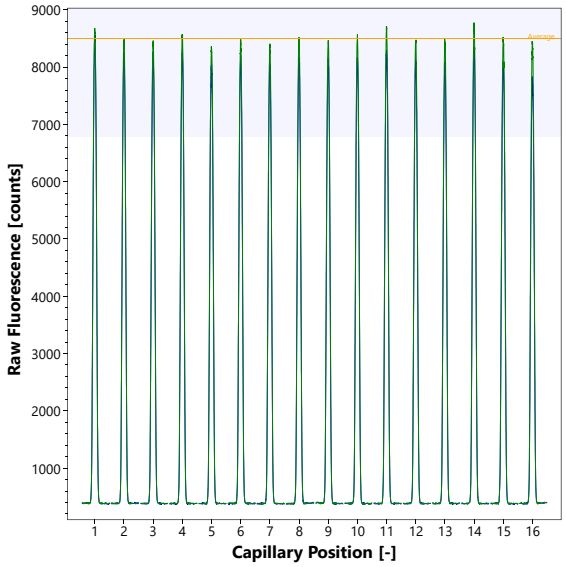
MO.Control v1.6 (NanoTemper Technologies GmbH)
https://nanotempertech.com/monolith-mo-control-software/

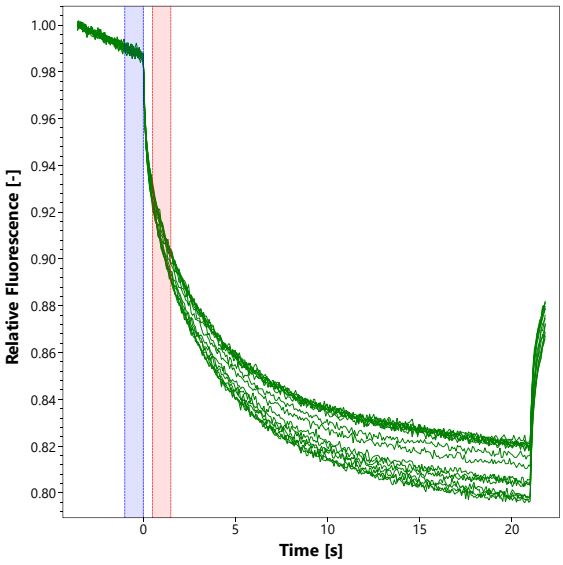
D3. MST Experiment (Concentrations/Temperature/MST Power/Excitation Power)

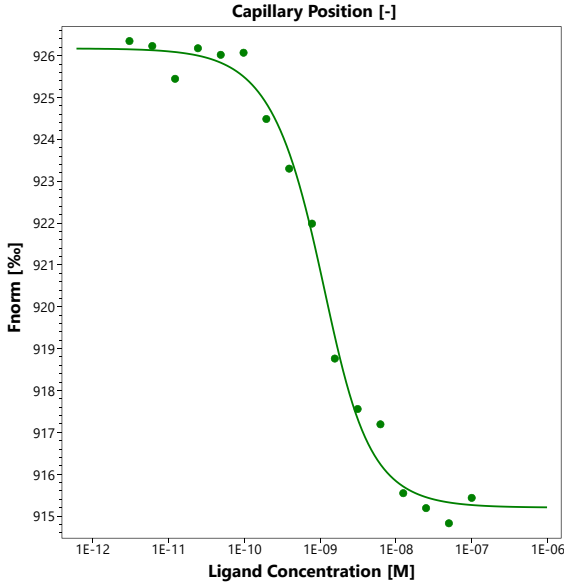
Phosphate-buffered saline (PBS), pH 7.4, 0.01% Pluronic® F-127
1 nM Fab | 100 nM – 3 pM DIG-DNA | 25°C | medium MST power | 40% excitation power

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

K_d = 0.66 nM







D5. Reference Results/Supporting Results

K_d = 0.91 ± 0.22 nM Surface-Plasmon Resonance (SPR)
Daugherty et al., Protein Engineering 11 (9), 825-832, (1998)

E. Contributors

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