Monolith Protocol MO-P-052

Anti-Digoxigenin, Fab fragments – Digoxigenin

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 20 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 2 µL of the 600 µM dye solution with 18 µL of Labeling Buffer NHS to obtain 20 µL of a 60 µM dye solution (3x protein concentration).
5. Mix Fab and dye in a 1:1 volume ratio (40 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 40 µL of the labeling reaction from step 5 to the center of the column and let sample enter the resin bed completely.
10. Add 550 µL of dilution buffer after the sample has entered and discard the flow through.
11. Place column in a new collection tube, add 400 µL of dilution buffer and collect the eluate.
12. Keep the labeled Fab (~1 µM) on ice in the dark.

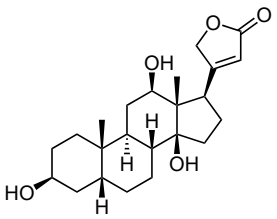
A10. Labeling Efficiency

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

Absorbance A_{280}	0.076	Protein concentration	~1.00 µM
Absorbance A_{650}	0.153	Degree-of-labeling (DOL)	~0.79

B1. Ligand/Non-Fluorescent Binding Partner

Digoxigenin (DIG)



B2. Molecule Class/Organism

Steroid

B3. Sequence/Formula

$C_{23}H_{34}O_5$

B4. Purification Strategy/Source

Sigma-Aldrich GmbH
D9026

B5. Stock Concentration/Stock Buffer

25 mg
Powdered

B6. Molecular Weight/Extinction Coefficient

390.51 Da

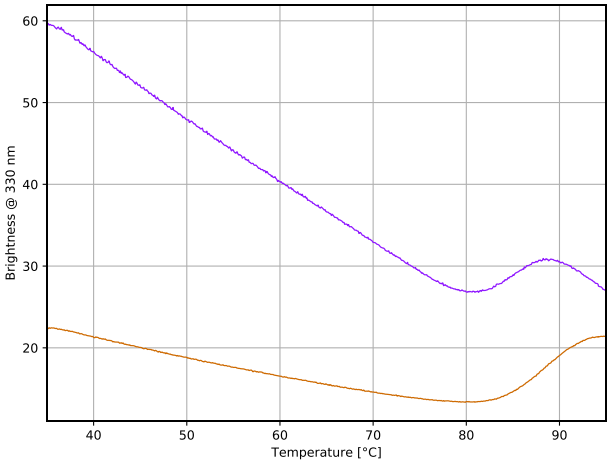
B7. Serial Dilution Preparation

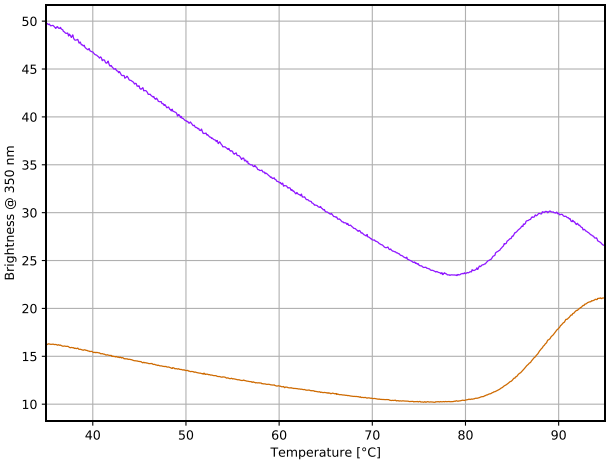
1. Dissolve 25 mg of DIG in 6.4 mL of DMSO to obtain a 10 mM DIG solution.
2. Add 78 µL of DMSO to 2 µL of 10 mM DIG to obtain 80 µL of a 250 µM DIG solution in DMSO.
3. Mix 2 µL of the 250 µM DIG solution with 98 µL of PBS to obtain 100 µL of a 5 µM DIG solution in PBS containing 2% DMSO.
4. Prepare a PCR-rack with 16 PCR tubes. Mix 4 µL of the 5 µM DIG solution with 36 µL of dilution buffer in tube **1**. Then, transfer 20 µL of dilution buffer into tubes **2** to **16**.
5. 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.
6. Mix 2 µL of labeled Fab (~1 µM) with 998 µL of dilution buffer to obtain 1 mL of a ~2 nM Fab solution.
7. Add 20 µL of this solution to each tube from **16** to **1** and mix by pipetting.
8. Incubate tubes for at least 30 minutes at room temperature in the dark before loading capillaries.

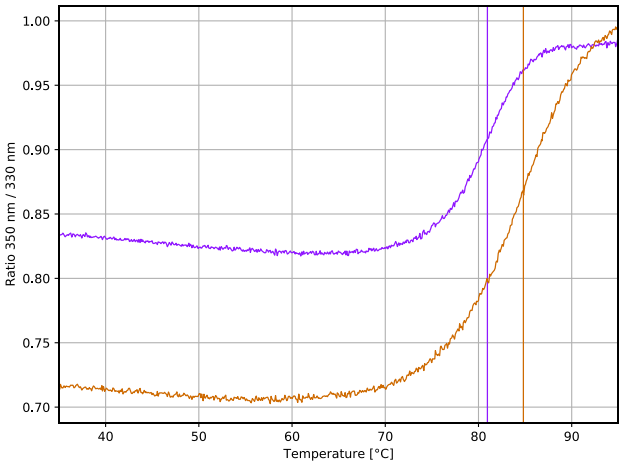
C. Applied Quality Checks

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

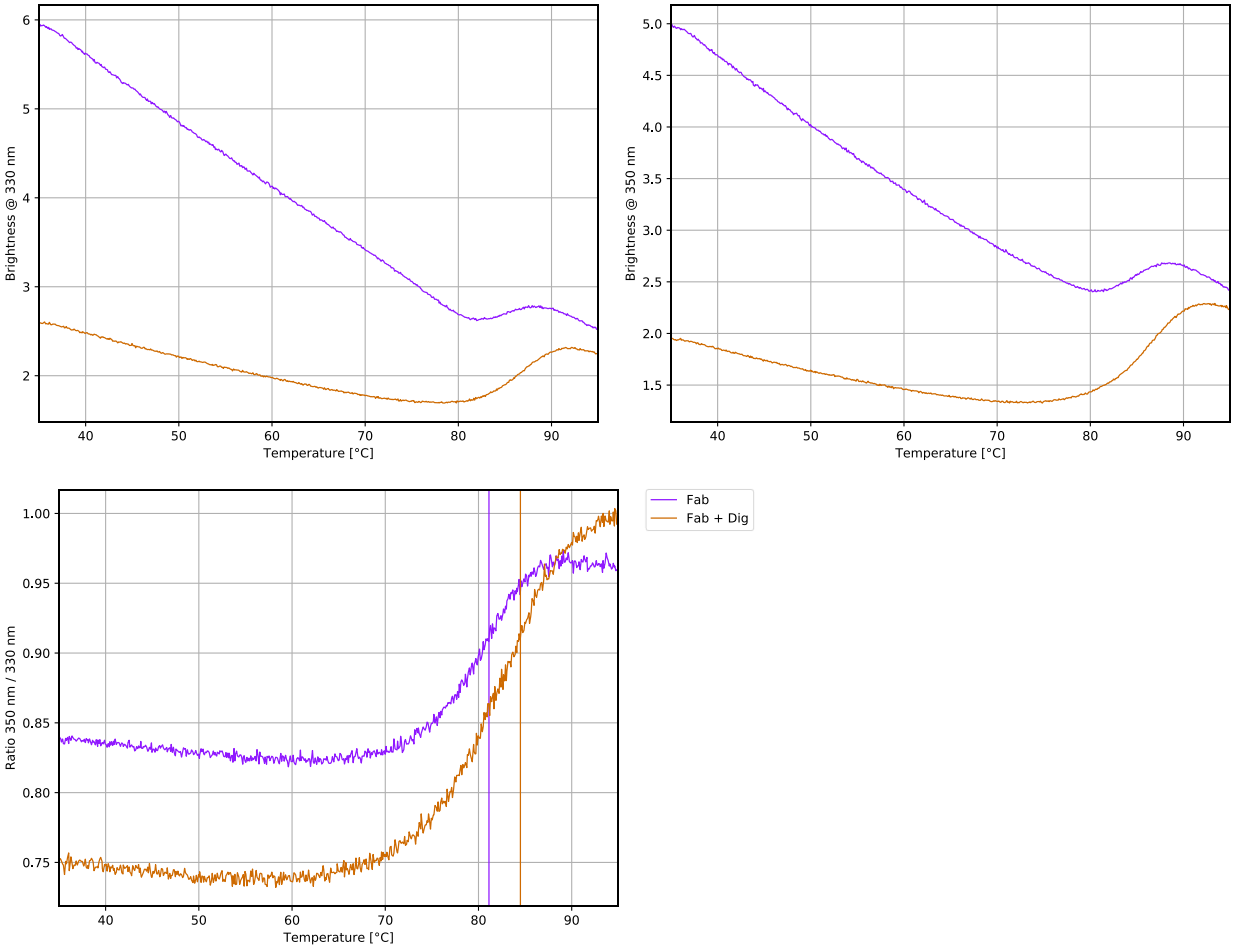
Fab	2 µL of 20 µM Fab + 8 µL of PBS	T_i = 81.0°C
Fab + Dig	2 µL of 20 µM Fab + 8 µL of 5 µM digoxigenin	T_i = 84.8°C







Fab (labeled)	8 µL of labeled Fab (~1µM) + 2 µL of PBS	T_i = 81.1°C
Fab (labeled) + Dig	8 µL of labeled Fab (~1µM) + 2 µL of 5 µM digoxigenin	T_i = 84.5°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

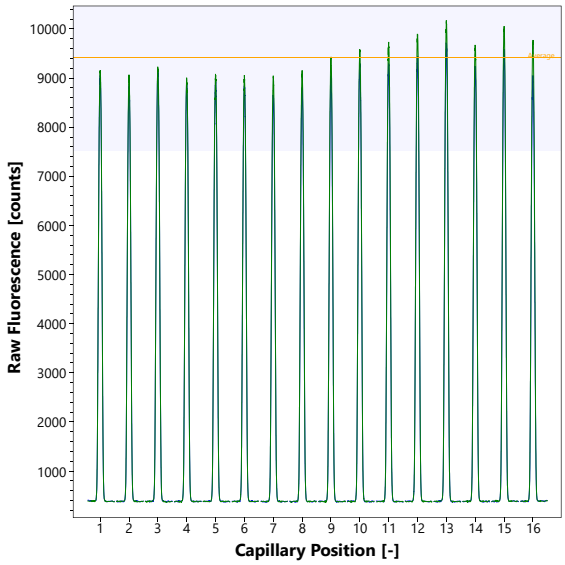
MO.Control v1.6 (NanoTemper Technologies GmbH)
https://nanotempertech.com/monolith-mo-control-software/

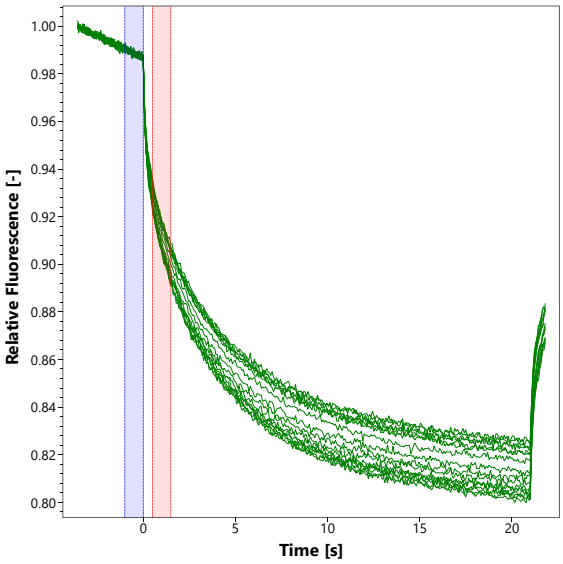
D3. MST Experiment (Concentrations/Temperature/MST Power/Excitation Power)

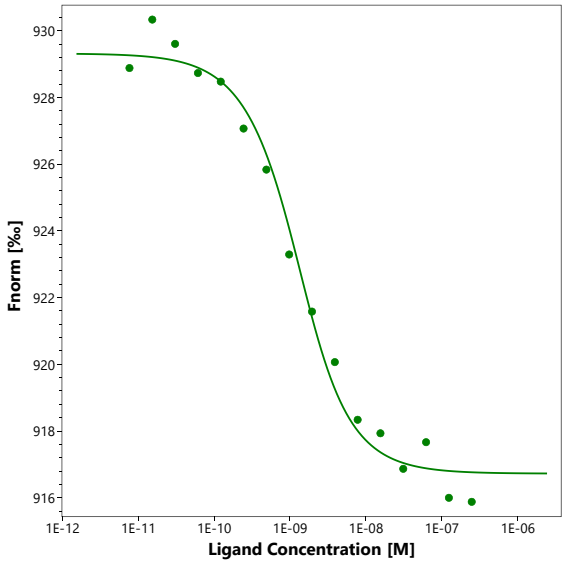
Phosphate-buffered saline (PBS), pH 7.4, 0.01% Pluronic® F-127
1 nM Fab | 250 nM – 7.6 pM DIG | 22°C | medium MST power | 40% excitation power

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

K_d = 0.81 ± 0.13 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

Andreas Langer[1]

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