Monolith Protocol MO-P-048

Mitochondrial Fission 1 Protein – 12 mer Peptide

Mitochondrial fission 1 protein (FIS1) is a component of the mitochondrial complex ARCosome, which promotes mitochondrial fission. FIS1 protein is involved in cell cycle and apoptosis and plays a role in several associated diseases, like diabetic cardiomyopathy, neurodegenerative diseases and cancer.

protein – peptide interaction | mitochondrial fission protein | FIS1

A1. Target/Fluorescent Molecule

Mitochondrial fission 1 protein (FIS1)
uniprot.org/uniprot/Q9Y3D6

A2. Molecule Class/Organism

Cytosolic domain of mitochondrial outer membrane surface protein
Homo sapiens (Human)

A3. Sequence/Formula

MEAVLNELVS VEDLLKFEKK FQSEKAAGSV SKSTQFEYAW CLVRSKYNDD IRKGIVLLEE LLPKGSKEEQ RDYVFYLAVG
NYRLKEYEKA LKYVRGLLQT EPQNNQAKEL ERLIDKAMKK DGLVG

A4. Purification Strategy/Source

Nickel chromatography followed by size-exclusion chromatography, > 95 %
Medical College of Wisconsin (John M. Egner)

A5. Stock Concentration/Stock Buffer

0.145 mg/mL | 10 µM
100 mM HEPES, pH 7.4, 200 mM NaCl, 1 mM DTT, 0.02 % sodium azide

A6. Molecular Weight/Extinction Coefficient

14.5 kDa
15,930 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

100 mM HEPES, pH 7.4, 200 mM NaCl, 1 mM DTT, 0.02 % sodium azide, 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. Prepare 100 µL of a 10 µM FIS1 solution.
2. Use the A-Column to perform a buffer exchange into Labeling Buffer NHS **supplemented** with 1 mM GSH.
   a. Invert A-Column to suspend slurry and twist off bottom (twist slightly in both directions).
   b. Loosen the cap of the column and place it in a 1.5 mL microcentrifuge collection tube.
   c. Centrifuge at **1500 × g** for **1 min** to remove excess liquid.
   d. Add 300 µL of Labeling Buffer NHS with GSH and centrifuge at **1500 × g** for **1 min** (3x).
   e. Place 100 µL of the 10 µM FIS1 solution in the center of the resin.
   f. Place the sample in a **new** microcentrifuge collection tube and centrifuge at **1500 × g** for **2 min**.

   The collected flow-through should yield around 100 µL of ~5 µM FIS1 (~50 % recovery).
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 with GSH to obtain 100 µL of a 60 µM dye solution (8x protein concentration).
5. Mix FIS1 and dye in a 1:1 volume ratio (200 µL final volume).
6. Incubate for 30 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 bed completely.
10. Add 400 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 FIS1 (~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}	0.021	Protein concentration	1.1 µM
Absorbance A_{650}	0.096	Degree-of-labeling (DOL)	0.45

B1. Ligand/Non-Fluorescent Binding Partner

12 mer peptide (pep2)

B2. Molecule Class/Organism

Peptide

B3. Sequence/Formula

N/A

B4. Purification Strategy/Source

Genscript peptide synthesis

B5. Stock Concentration/Stock Buffer

5.66 mM
100 mM HEPES, pH 7.4, 200 mM NaCl, 1 mM DTT, 0.02 % sodium azide

B6. Molecular Weight/Extinction Coefficient

N/A

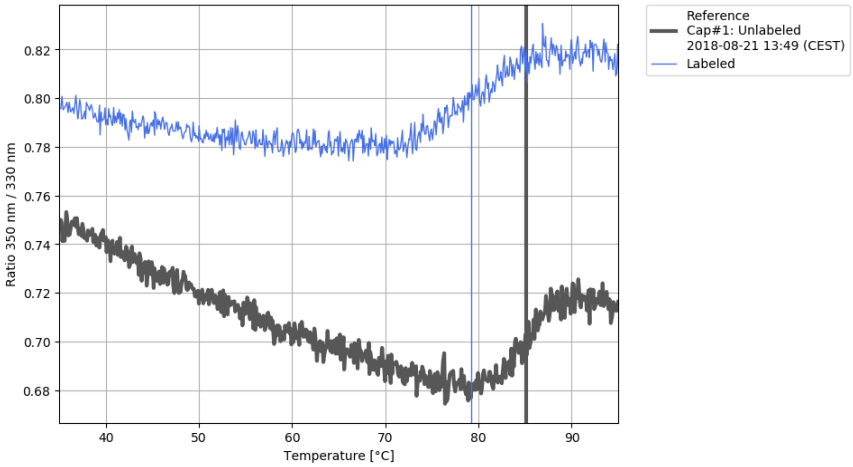
B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Transfer 7 µL of the 5.66 mM pep2 stock solution into tube **1** and mix with 13 µL of dilution buffer to obtain 20 µL of a 2 mM solution. 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 2 µL of labeled FIS1 with 198 µL of dilution buffer to obtain 200 µL of ~10 nM FIS1.
4. Add 10 µL of labeled FIS1 (~10 nM) to each tube from **16** to **1** and mix by pipetting.
5. Incubate for 30 minutes at room temperature in the dark before loading capillaries.

C. Applied Quality Checks

Validation of structural integrity of labeled FIS1 using Tycho NT.6:
nanotempertech.com/tycho

Unlabeled	10 µL of FIS1 in dilution buffer (~5 µM)	T_i = 85.1°C
Labeled	10 µL of B-Column eluate (~1.1 µM)	T_i = 79.2°C



D1. MST System/Capillaries

Monolith NT.115^{Pico} Red (NanoTemper Technologies GmbH)
Premium Capillaries Monolith NT.115 (MO-K025, NanoTemper Technologies GmbH)

D2. MST Software

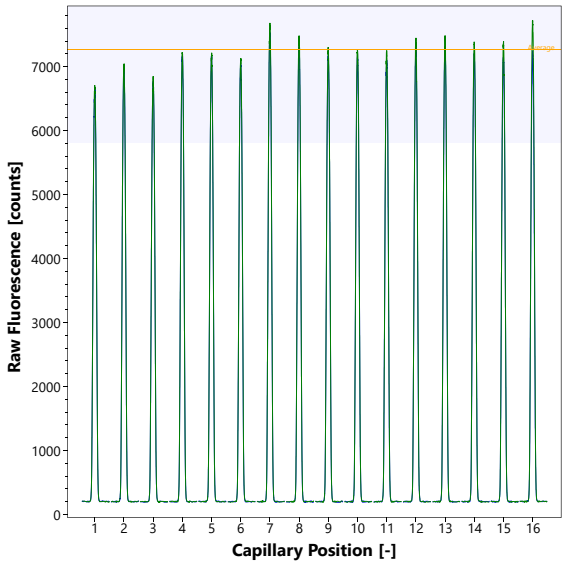
MO.Control v1.6 (NanoTemper Technologies GmbH)
https://nanotempertech.com/monolith-mo-control-software/

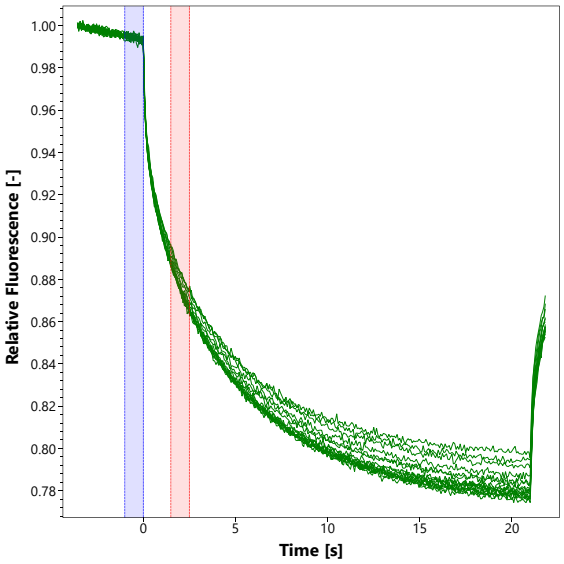
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

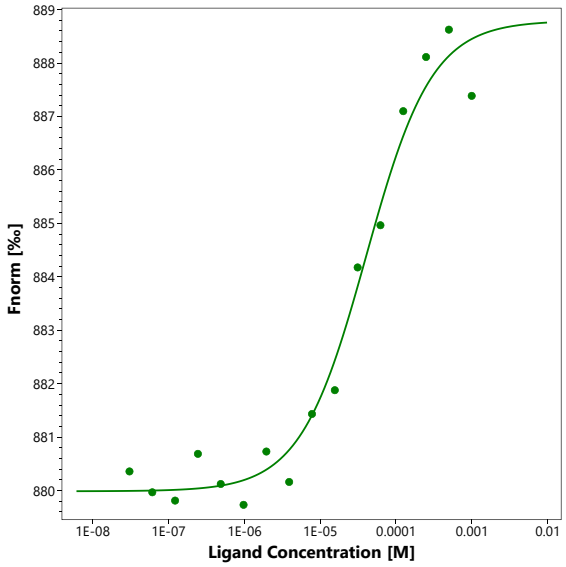
100 mM HEPES, pH 7.4, 200 mM NaCl, 1 mM DTT, 0.02 % sodium azide, 0.05 % TWEEN® 20
5 nM FIS1 | 1 mM – 30.5 nM pep2 | 25°C | medium MST power | 20% excitation power

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

K_d = 40.6 µM







D5. Reference Results/Supporting Results

K_d = 200 µM 2D-protein detected NMR titration experiments
Inhouse-measurements at Medical College of Wisconsin

E. Contributors

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