Monolith Protocol MO-P-029

Human NFS1 – ISCU2

Iron–sulfur (Fe/S) clusters are essential protein cofactors crucial for many cellular functions including DNA maintenance, protein translation and energy conversion. De novo Fe/S cluster synthesis in human cells occurs on the mitochondrial scaffold protein ISCU2 and requires cysteine desulfurase NFS1, ferredoxin FDX2, frataxin FXN, and the small proteins ISD11 and ACP.

protein – protein interaction | iron-sulfur (Fe/S) clusters | mitochondrial scaffold protein | cysteine desulfurase

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A1. Target/Fluorescent Molecule

NFS1 $(NFS1\text{-}ISD11\text{-}ACP)_2$ complex, $(NIA)_2$
uniprot.org/uniprot/Q9Y697 | uniprot.org/uniprot/Q9HD34 | uniprot.org/uniprot/P0A6A8

A2. Molecule Class/Organism

Cysteine desulfurase (transaminase)
Homo sapiens (Human) – NFS1, ISD11 | *Escherichia coli* – ACP

A3. Sequence/Formula

NFS1

MSLRPLYMDV QATTPLDPRV LDAMLPYLIN YYGNPHSRTH AYGWESEAAM ERARQQVASL IGADPREIIF TSGATESNNI AIKGVARFYR SRKKHLITTQ TEHKCVLDSC RSLEAEGFQV TYLPVQKSGI IDLKELEAAI QPDTSLVSVM TVNNEIGVKQ PIAEIGRICS SRKVYFHTDA AQAVGKIPLD VNDMKIDLMS ISGHKIYGPK GVGAIYIRRR PRVRVEALQS GGGQERGMRS GTVPTPLVVG LGAACEVAQQ EMEYDHKRIS KLSERLIQNI MKSLPDVVMN GDPKHHYPGC INLSFAYVEG ESLLMALKDV ALSSGSACTS ASLEPSYVLR AIGTDEDLAH SSIRFGIGRF TTEEEVDYTV EKCIQHVKRL REMSPLWEMV QDGIDLKSKW TQH

ISD11

MGSSHHHHHH GSPTTENLYF QGHNMAASSR AQVLALYRAM LRESKRFSAY NYRTYAVRRI RDAFRENKNV KDPVEIQTLV NKAKRDLGVI RRQVHIGQLY STDKLIIENR DMPRT

ACP

MSTIEERVKK IIGEQLGVKQ EEVTNNASFV EDLGADSLDT VELVMALEEE FDTEIPDEEA EKITTVQAAI DYINGHQA

A4. Purification Strategy/Source

NFS1/ISD11 was co-expressed and purified from E. coli One Shot BL21 Star (DE3) cells (ThermoFisher Scientific) in complex with endogenous ACP using Ni-affinity chromatography (His-tag on ISD11).

A5. Stock Concentration/Stock Buffer

37 mg/mL | 250 µM
35 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5% glycerol

A6. Molecular Weight/Extinction Coefficient

146.7 kDa

A7. Dilution Buffer

35 mM KP_i, pH 7.4, 150 mM NaCl

A8. Labeling Strategy

Monolith Protein Labeling Kit RED – NHS (MO-L001, NanoTemper Technologies GmbH)
1* Labeling Buffer NHS | 1* 10 µg NT-647-NHS dye | 1* B-Column

A9. Labeling Procedure

1. Add 92 µL of Labeling Buffer NHS to 8 µL of 250 µM $(NIA)_2$ to obtain 100 µL of a 20 µM solution.
2. Add 30 µL of DMSO to 10 µg NT-647-NHS dye to obtain a ~470 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
3. Mix 12.8 µL of the 600 µM dye solution with 87.2 µL of Labeling Buffer NHS to obtain 100 µL of a 60 µM dye solution (3x protein concentration).
4. Mix $(NIA)_2$ and dye in a 1:1 volume ratio (200 µL final volume, 6.4% final DMSO concentration).
5. Incubate for 1 hr at room temperature in the dark.
6. 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.
7. 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.
8. Add 200 µL of the labeling reaction from step 5 to the center of the column and let sample enter the bed completely.
9. Add 400 µL of dilution buffer after the sample has entered and discard the flow through.
10. Place column in a new collection tube, add 500 µL of dilution buffer and collect the eluate.
11. Keep the labeled $(NIA)_2$ (~4 µM) on ice in the dark.

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

ISCU2

B2. Molecule Class/Organism

Mitochondrial scaffold protein
Homo sapiens (Human)

B3. Sequence/Formula

MAYHKKVVDH YENPRNVGSL DKTSKNVGTG LVGAPACGDV MKLQIQVDEK GKIVDARFKT FGCGSAIASS SLATEWVKGK
TVEEALTIKN TDIAKELCLP PVKLHCSMLA EDAIKAALAD YKLKQEPKKG EAEKKLEHHH HHH

B4. Purification Strategy/Source

ISCU2 was expressed and purified from in E. coli One Shot BL21 Star (DE3) cells (ThermoFisher Scientific) using Ni-affinity chromatography.

Prior to MST measurements ISCU2 was treated with 5 mM DTT, 1 mM KCN, and 10 mM EDTA and subsequently gel filtered using a HiLoad 16/60 Superdex 75 PG (GE Healthcare).

B5. Stock Concentration/Stock Buffer

87 mg/mL | 6.3 mM
35 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5% glycerol

B6. Molecular Weight/Extinction Coefficient

13.8 Da
9,970 $M^{-1}cm^{-1}$ (ε_{280})

B7. Serial Dilution Preparation

1. Add 95.8 µL of dilution buffer to 4.2 µL of the 6.3 mM ISCU2 stock to obtain 100 µL of a 266 µM solution.
2. Prepare a PCR-rack with 16 PCR tubes. Transfer 30 µL of the 266 µM ISCU2 solution into tube **1**. Then, transfer 15 µL of dilution buffer into tubes **2** to **16**.
3. Prepare a 1:1 serial dilution by transferring 15 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 15 µL from tube **16** to get an equal volume of 15 µL for all samples.
4. Mix 20 µL of labeled $(NIA)_2$ with 80 µL of dilution buffer to obtain 100 µL of ~800 nM $(NIA)_2$.
5. Add 5 µL of labeled $(NIA)_2$ (~800 nM) to each tube from **16** to **1** and mix by pipetting.
6. Incubate for 5 minutes at room temperature in the dark before loading capillaries.

C. Applied Quality Checks

CD spectroscopy

D1. MST System/Capillaries

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

D2. MST Software

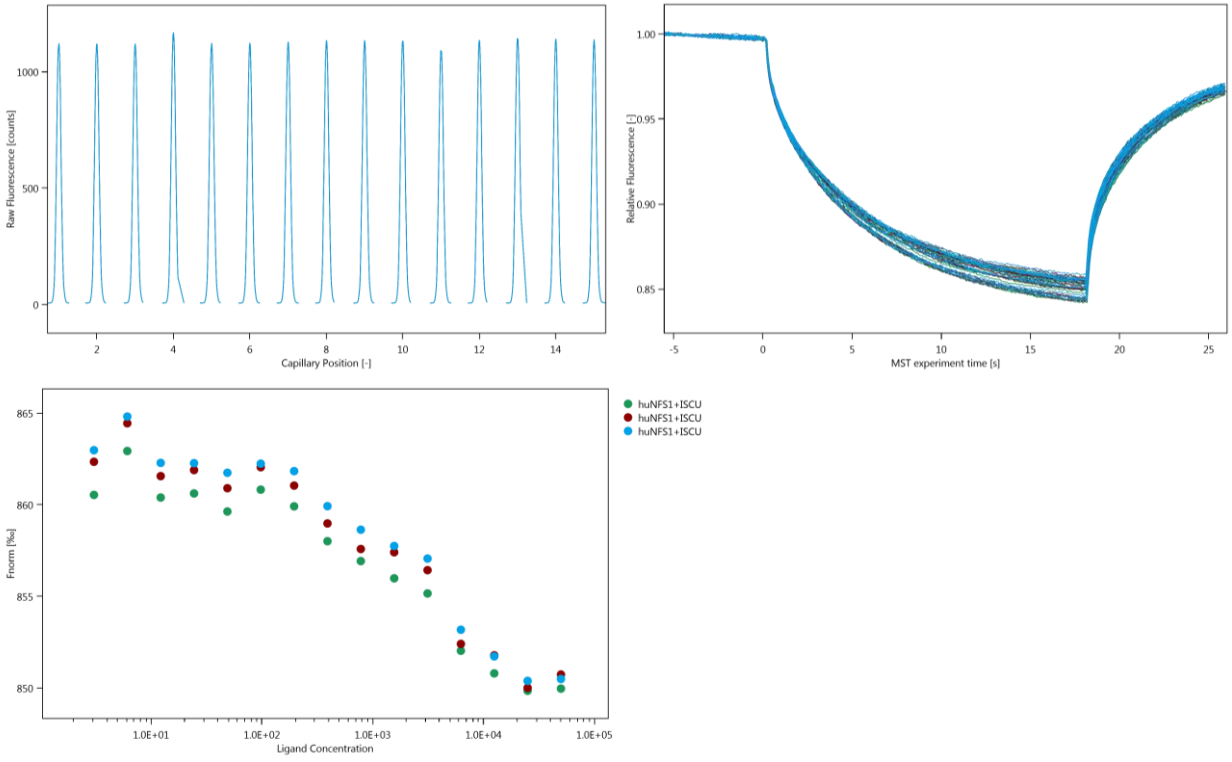
MO.Affinity Analysis v2.3 (NanoTemper Technologies GmbH)

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

35 mM KP_i, pH 7.4, 150 mM NaCl
200 nM $(NIA)_2$ | 200 µM – 3.05 nM ISCU2 | 22°C | 75% MST Power | 50% excitation power

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

K_d = 1.7 µM Boniecki et al., Nature Communications, 8, 1287 (2017)



D5. Reference Results/Supporting Results

K_d = 1.84 µM BioLayer Interferometry (BLI) – Octet K2

E. Contributors

Sven Freibert[1], Nina Schlinck[2]

[1] Institute for Cytobiology and Cytopathology, University of Marburg, Marburg, Germany
[2] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com