Monolith X Protocol MOX-P-116

Anti-6x-His Tag Monoclonal Antibody – p38-alpha

The 6x His tag is a synthetic oligo peptide consisting of 6 consecutive histidine residues (HHHHHH). The His tag is commonly expressed as a tag at either N-or C-terminal regions of recombinant proteins to allow isolation or purification by immobilized metal affinity chromatography. His tag-specific antibodies are used to facilitate detection or immunoprecipitation of His-tagged proteins. In this protocol, the interaction between the anti-His_6 antibody HIS.H8 and His_6-tagged mitogen-activated protein kinase 14 (p38-α) is measured.

protein – protein | antibody

A1. Target/Fluorescent Molecule

6x-His Tag Monoclonal Antibody (HIS.H8)

A2. Molecule Class/Organism

Monoclonal antibody

A3. Sequence/Formula

N/A

A4. Purification Strategy/Source

Invitrogen
MA1-21315

A5. Stock Concentration/Stock Buffer

1 mg/mL | 6.67 µM

A6. Molecular Weight/Extinction Coefficient

150 kDa
200,000 $M^{-1}cm^{-1}$ (ε_{280})[1]

A7. Dilution Buffer

20 mM HEPES, pH 7.4, 150 mM NaCl, 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

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[1] Estimated extinction coefficient for an antibody.

A9. Labeling Procedure

1. Prepare 60 µL of a 6.67 µM antibody solution.
2. Add 25 µL of DMSO to 10 µg RED-NHS 2nd Generation dye to obtain a ~600 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
3. Mix 4 µL of the 600 µM dye solution with 56 µL Labeling Buffer NHS to obtain 60 µL of a 40 µM dye solution (6x protein concentration).
4. Mix antibody and dye in a 1:1 volume ratio (120 µL final volume, ~3.3% final DMSO concentration).
5. Incubate for 30 minutes 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 120 µL of the labeling reaction from step 5 to the center of the column and let sample enter the resin bed completely.
9. Add 500 µL of dilution buffer after the sample has entered and discard the flow through.
10. Place column in a new collection tube, add 400 µL of dilution buffer and collect the eluate.
11. Centrifuge the eluate at 15,000 rpm and 4°C for 15 minutes to remove aggregates. Then, carefully transfer the supernatant into a fresh tube and mix well by pipetting.
12. Prepare 10 µL aliquots of the labeled antibody (~1 µM) and store at -80°C.

A10. Labeling Efficiency

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

Absorbance A_{280}	0.157	Protein concentration	~0.73 µM
Absorbance A_{650}	0.253	Degree-of-labeling (DOL)	~1.77

B1. Ligand/Non-Fluorescent Binding Partner

Mitogen-activated protein kinase 14 (p38-α)
uniprot.org/uniprot/Q16539

B2. Molecule Class/Organism

Mitogen-activated protein kinase (MAP kinase)
Homo sapiens (Human)

B3. Sequence/Formula

MSYYHHHHHH DYDIPTTENL YFQGAMWMSQ ERPTFYRQEL NKTIWEVPER YQNLSPVGSG AYGSVCAAFD TKTGLRVAVK
KLSRPFQSII HAKRTYRELR LLKHMKHENV IGLLDVFTPA RSLEEFNDVY LVTHLMGADL NNIVKCQKLT DDHVQFLIYQ
ILRGLKYIHS ADIIHRDLKP SNLAVNEDCE LKILDFGLAR HTDDEMTGYV ATRWYRAPEI MLNWMHYNQT VDIWSVGCIM
AELLTGRTLF PGTDHIDQLK LILRLVGTPG AELLKKISSE SARNYIQSLT QMPKMNFANV FIGANPLAVD LLEKMLVLDS
DKRITAAQAL AHAYFAQYHD PDDEPVADPY DQSFESRDLL IDEWKSLTYD EVISFVPPPL DQEEMES

B4. Purification Strategy/Source

Expressed in E. coli BL21 (DE3), Lot PC11655-3, Construct CJA3
Crelux GmbH

B5. Stock Concentration/Stock Buffer

2.2 mg/mL | 50 µM
25 mM HEPES, pH 7.4, 50 mM NaCl, 10 mM DTT, 1 mM EDTA

B6. Molecular Weight/Extinction Coefficient

44.7 kDa
50,100 $M^{-1}cm^{-1}$ (ε_{280})

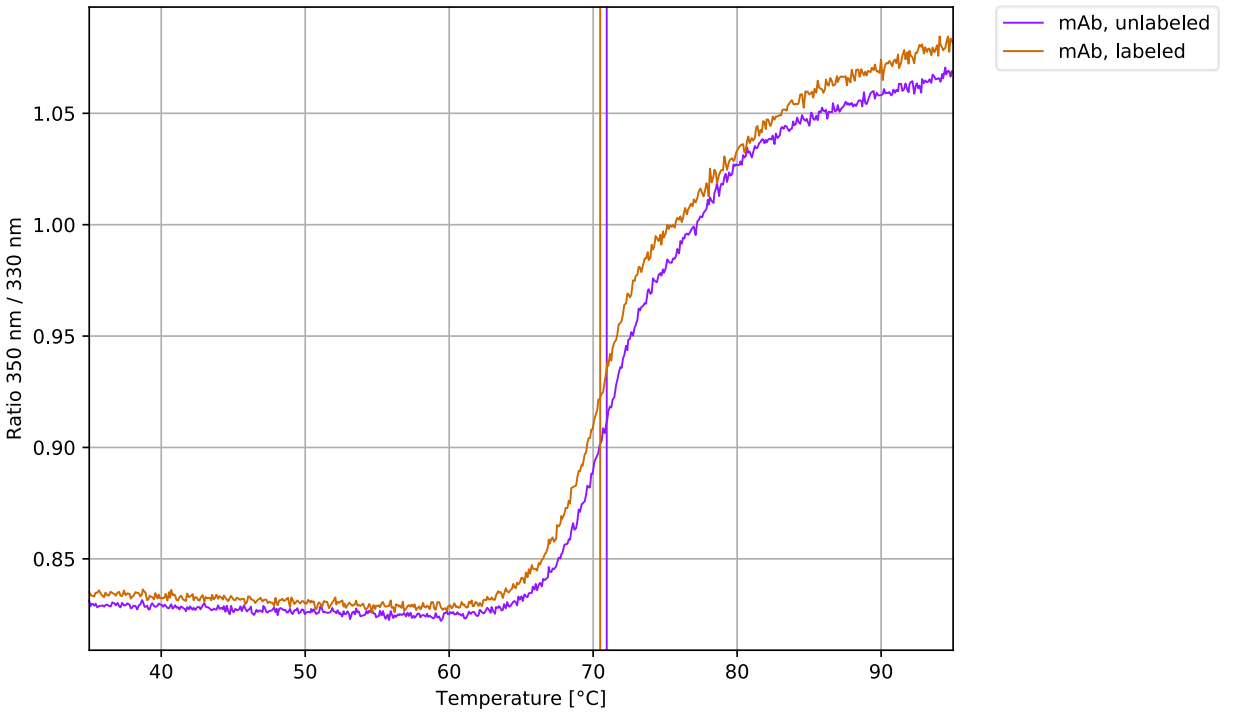
B7. Serial Dilution Preparation

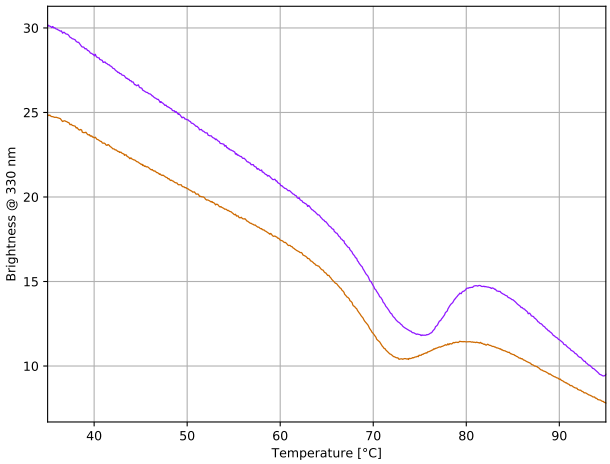
1. Dilute p38-α in dilution buffer for a final concentration of 500 nM.
2. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of 500 nM p38-α into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
3. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. 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.
4. Mix 2 µL of the labeled antibody (~1 µM) with 398 µL of dilution buffer to obtain 400 µL of a ~5 nM antibody solution.
5. Add 10 µL of labeled antibody (~5 nM) to each tube from **16** to **1** and mix by pipetting.
6. Incubate for 30 minutes at room temperature in the dark before loading capillaries.

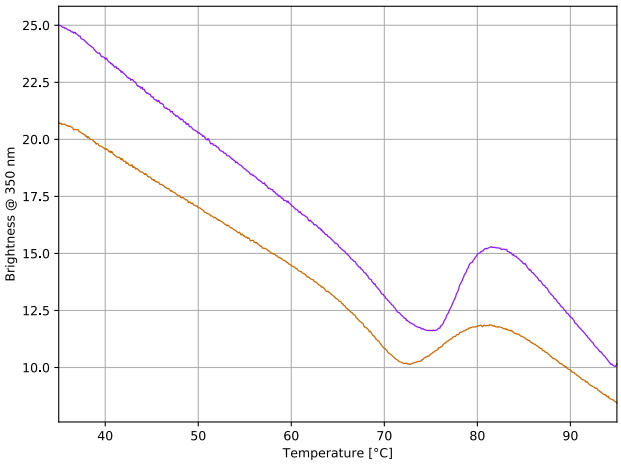
C. Tycho

Validation of structural integrity of labeled 6x-His Tag Monoclonal Antibody (HIS.H8) using Tycho NT.6:
nanotempertech.com/tycho

mAb, unlabeled	10 µL of 1 µM HIS.H8	T_i = 70.9°C
mAb, labeled	10 µL of B-Column eluate (~1 µM)	T_i = 70.5°C







D1. Monolith System/Capillaries

Monolith X (NanoTemper Technologies GmbH)
Monolith Premium Capillaries (MO-K025, NanoTemper Technologies GmbH)

D2. Monolith Software

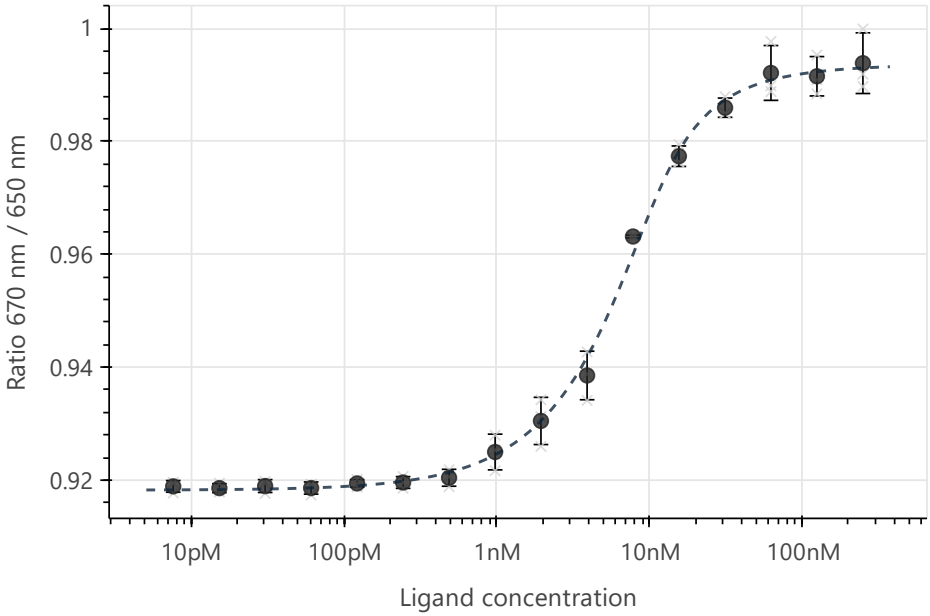
MO.Control v2.4.2 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

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

20 mM HEPES, pH 7.4, 150 mM NaCl, 0.01% Pluronic® F-127
2.5 nM 6x-His Tag Monoclonal Antibody (HIS.H8)[2] | 250 nM – 7.6 pM p38-α | 25°C | 100% excitation power

D4. Monolith Results (Dose Response)

K_d = 2.04 ± 0.27 nM (S/N = 54.1)



D5. Reference Results/Supporting Results

N/A

E. Contributors

Andreas Langer[3]

[2] The target concentration needs to be set to 5 nM due to the two binding sites of the antibody.
[3] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com