Monolith Protocol MO-P-030

CHP3 – NHE1

The Na^+/H^+ exchanger NHE1 is critical for cell vitality as it controls intracellular pH and cell volume. Its functionality is influenced by calcineurin B homologous proteins (CHPs). The human isoform CHP3 is important for transport of NHE1 to the plasma membrane and for its activity.

protein – protein interaction | sodium/proton exchanger | calcium-binding EF-hand protein

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A1. Target/Fluorescent Molecule

Calcineurin B homologous protein 3 (CHP3)
uniprot.org/uniprot/Q96BS2

A2. Molecule Class/Organism

Calcium-binding EF-hand protein
Homo sapiens (Human)

A3. Sequence/Formula

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MKHHHHHHPM SDYDIPTTEN LYFQGAGSMK QGAAHSASEE VRELEGKTGF SSDQIEQLHR RFKQLSGDQP TIRKENFNNV
PDLELNPIRS KIVRAFFDNR NLRKGPSGLA DEINFEDFLT IMSYFRPIDT TMDEEQVELS RKEKLRFLFH MYDSDSDGRI
TLEEYRNVVE ELLSGNPHIE KESARSIADG AMMEAASVCM GQMEPDQVYE GITFEDFLKI WQGIDIETKM HVRFLNMETM
ALCH
```

A4. Purification Strategy/Source

CHP3 was produced in E. coli BL21 (DE3) cells and purified using Ni-affinity chromatography and SEC.

A5. Stock Concentration/Stock Buffer

10 mg/mL | 350 µM
20 mM HEPES, pH 7.2, 150 mM NaCl, 20 mM DTT, 1 M Sucrose

A6. Molecular Weight/Extinction Coefficient

28 kDa
14,440 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

20 mM HEPES, pH 7.2, 150 mM NaCl, 10 mM $MgCl_2$, 5 mM TCEP[1], 0.05% TWEEN® 20

[1] Since TCEP solutions are very acidic (~pH 2.5), all TCEP stock solutions were brought to pH 7.2 with NaOH.

A8. Labeling Strategy

Monolith Protein Labeling Kit RED – NHS (MO-L001, NanoTemper Technologies GmbH)
1* A-Column | 2* 10 µg RED-NHS dye | 1* B-Column

A9. Labeling Procedure

1. Thaw 100 µL of the 350 µM CHP3 stock.
2. Prepare a buffer solution containing 50 mM HEPES, pH 7.6, 150 mM NaCl, 1 mM TCEP (Labeling Buffer).
3. Use the A-Column to perform a buffer exchange into Labeling Buffer.
   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 and centrifuge at **1500 × g** for **1 min** (3x).
   e. Place 100 µL of the 350 µM CHP3 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 ~250 µM CHP3 (70 – 80% recovery).
4. Determine the concentration of CHP3 in Labeling Buffer spectroscopically (NanoDrop™) and subsequently dilute it to 40 µM with Labeling Buffer.
5. Add 50 µL of Labeling Buffer each to two vials of 10 µg RED-NHS dye to obtain a 280 µM solution, respectively. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved. Then, combine both dye solutions to obtain a total volume of 100 µL of dye solution.
6. Mix 100 µL of the 280 µM dye solution with 100 µL of 40 µM CHP3 (200 µL final volume, 7x protein concentration).
7. Incubate for 30 minutes at room temperature in the dark.
8. 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.
9. 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.
10. Add 200 µL of the labeling reaction from step 7 to the center of the column and let sample enter the bed completely.
11. Add 300 µL of dilution buffer after the sample has entered and discard the flow through.
12. Add 600 µL of dilution buffer and collect the eluate three drops at a time (~100 µl fractions) in 0.6 mL tubes.
13. Measure OD_{280} and OD_{650} of each fraction, then combine fractions containing protein.
14. Keep the labeled CHP3 (~20 µM) on ice in the dark.

A10. Labeling Efficiency

Measurement of protein concentration and degree of labeling (DOL) using a Cary 4000 UV-Vis spectrophotometer (Agilent):
nanotempertech.com/dol-calculator

Absorbance A_{280}	0.31	Protein concentration	19.4 µM
Absorbance A_{650}	1.06	Degree-of-labeling (DOL)	0.22

B1. Ligand/Non-Fluorescent Binding Partner

CBD region (N-terminal region of the regulatory domain) of NHE1 (MBP-CBD)

B2. Molecular Class/Organism

Sodium/proton exchanger 1 (NHE1) +
Homo sapiens (Human) – GC01M027109/Gene ID: 6548

Maltose-binding protein
Escherichia coli – Gene ID: 948538

B3. Sequence/Formula

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MKIEEGKLVI WINGDKGYNG LAEVGKKFEK DTGIKVTVEH PDKLEEKFPQ VAATGDGPDI IFWAHDRFGG YAQSGLLAEI
TPDKAFQDKL YPFTWDAVRY NGKLIAYPIA VEALSLIYNK DLLPNPPKTW EEIPALDKEL KAKGKSALMF NLQEPYFTWP
LIAADGGYAF KYENGKYDIK DVGVDNAGAK AGLTFLVDLI KNKHMNADTD YSIAEAAFNK GETAMTINGP WAWSNIDTSK
VNYGVTVLPT FKGQPSKPFV GVLSAGINAA SPNKELAKEF LENYLLTDEG LEAVNKDKPL GAVALKSYEE ELVKDPRIAA
TMENAQKGEI MPNIPQMSAF WYAVRTAVIN AASGRQTVDE ALKDAQTNSS SNNNNNNNNN NLGENLYFQG GVDLLAVKKK
QETKRSINEE IHTQFLDHLL TGIEDICGHY G
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B4. Purification Strategy/Source

MBP-CBD was produced and purified from E. coli BL21 (DE3) cells using Ni-affinity chromatography and SEC.

B5. Stock Concentration/Stock Buffer

10 mg/mL | 205 µM
20 mM HEPES, pH 7.2, 150 mM NaCl, 1 mM TCEP, 1 M Sucrose

B6. Molecular Weight/Extinction Coefficient

48 kDa
69,330 $M^{-1}cm^{-1}$ (ε_{280})

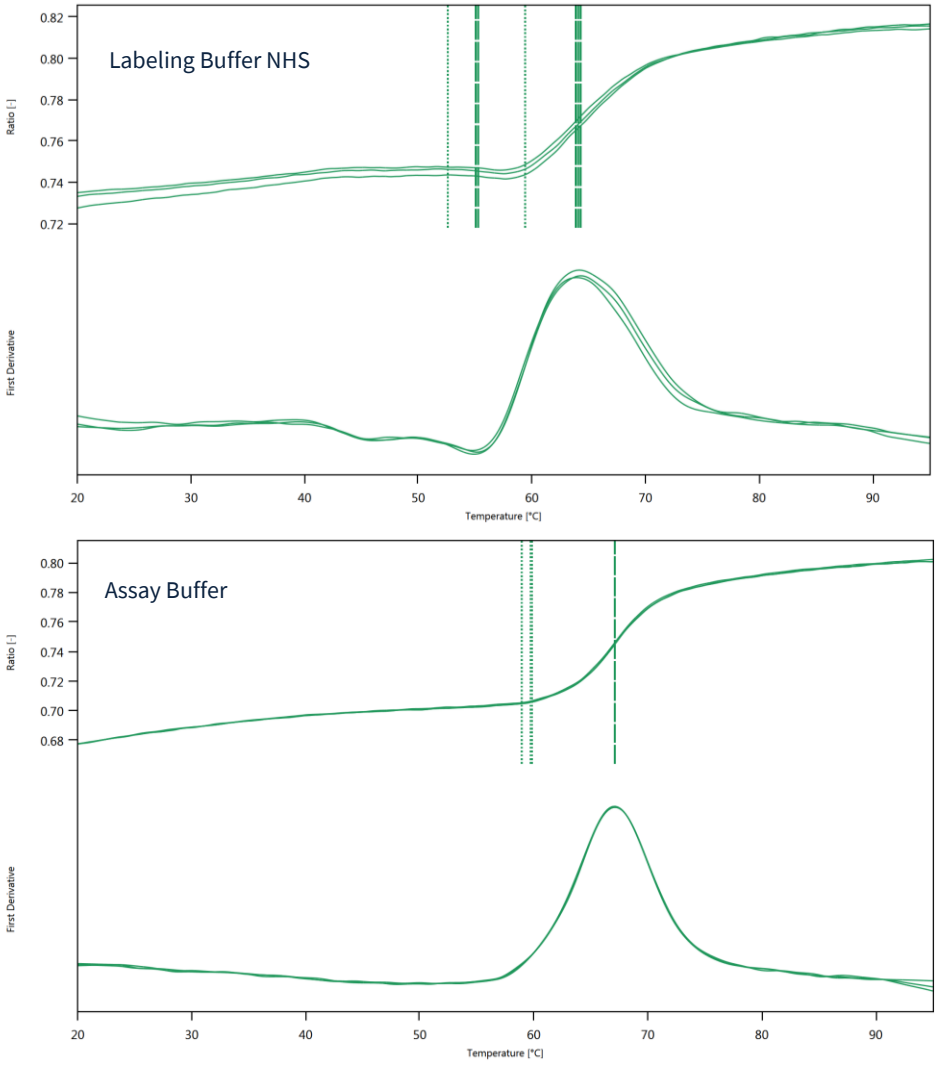
B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Mix 1.95 µL of the 205 µM MBP-CBD stock solution with 18 µL of dilution buffer in tube **1**. 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 CHP3 (~20 µM) with 2 mL of dilution buffer to obtain a ~20 nM CHP3 solution.
4. Add 10 µL of 20 nM labeled CHP3 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 CHP3 using Prometheus NT.48:
nanotempertech.com/prometheus

Labeling Buffer NHS	3 mg/mL CHP3; heating rate of 1°C/min	T_m = 63.9°C
Assay Buffer	3 mg/mL CHP3; heating rate of 1°C/min	T_m = 67.1°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

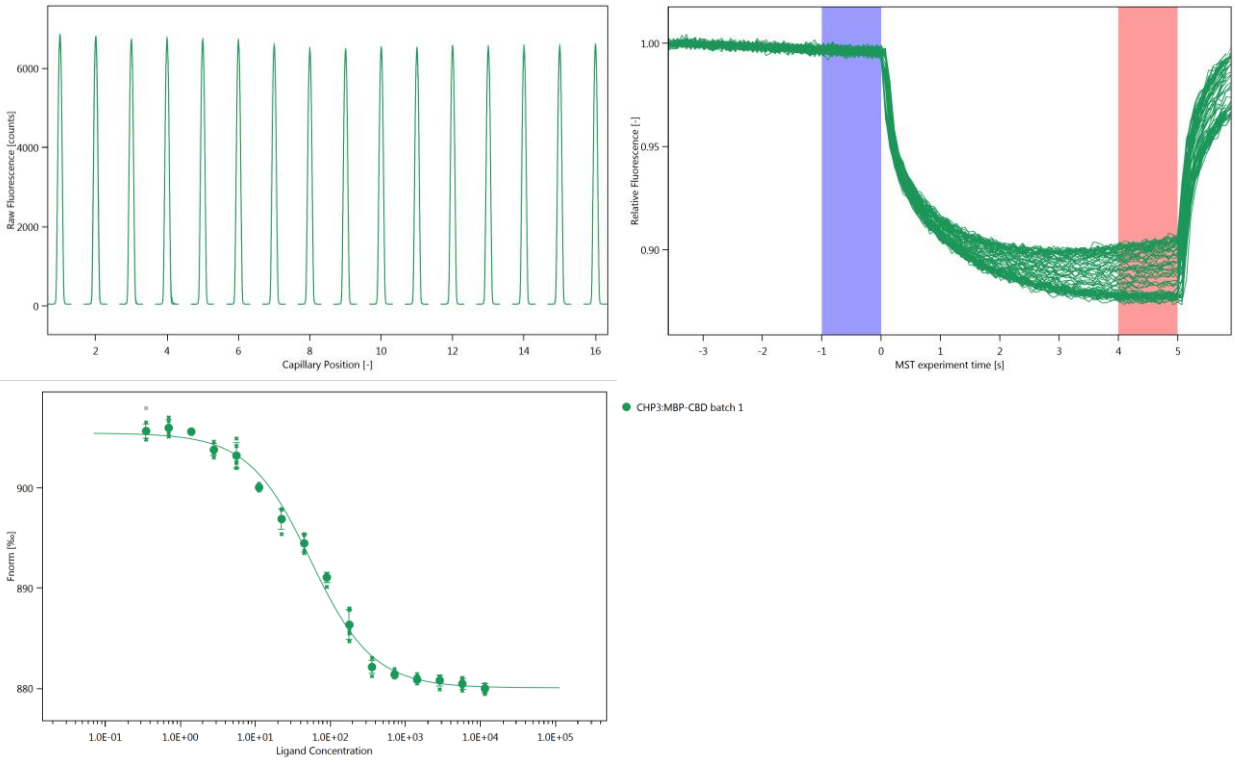
MO.Control v1.6 | MO.Affinity Analysis v2.3 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

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

20 mM HEPES, pH 7.2, 150 mM NaCl, 10 mM $MgCl_2$, 5 mM TCEP, 0.05% TWEEN® 20
10 nM CHP3 | 10 µM – 0.3 nM BP-CBD | 22°C | medium MST power | 10% excitation power

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

K_d = 55.5 ± 6.8 nM Fuchs et al., Scientific Reports 8:14837 (2018)



D5. Reference Results/Supporting Results

K_d = 3 nM Isothermal Titration Calorimetry (ITC)
Fuchs et al., Scientific Reports 8:14837 (2018)

E. Contributors

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