Monolith X Protocol MOX-P-105

Maltose Binding Protein (biotinylated) – Maltose

Maltose binding protein (MBP) is part of the periplasmic transport system of *Escherichia coli* and involved in the transport of maltose into the bacterium. It binds the disaccharide once it has crossed the outer membrane, and then assists its translocation across the inner membrane. Additionally, it is often used as a fusion tag for protein purification or solubilization. In this protocol, biotinylated MBP with an AVI-tag is fluorescently labeled using Nanotemper Technologies' Biotinylated Target Labeling Kit (NT-L020).

protein – small molecule | carbohydrate | conformational change

---

A1. Target/Fluorescent Molecule

Maltose/maltodextrin-binding periplasmic protein (MBP)
uniprot.org/uniprot/P0AEX9

A2. Molecule Class/Organism

Periplasmic protein
Escherichia coli

A3. Sequence/Formula

MKTEEGKLVI 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 ELAKDPRIAA
TMENAQKGEI MPNIPQMSAF WYAVRTAVIN AASGRQTVDE ALKDAQTNSS SGSLSTPPTP SPSTPPTGLN DIFEAQKIEW
HE[1]

A4. Purification Strategy/Source

Avidity
BIS-300 positive control

A5. Stock Concentration/Stock Buffer

1 mg/mL | 22 µM

A6. Molecular Weight/Extinction Coefficient

44 kDa
71,850 $M^{-1}cm^{-1}$ (ε_{280})

[1] AVI-tag colored in red.

A7. Dilution Buffer

20 mM HEPES, pH 7.4, 150 mM NaCl, 0.01% Pluronic F-127®

A8. Labeling Strategy

Biotinylated Target Labeling Kit (NT-L020 NanoTemper Technologies GmbH), 1* 20 pmol labeling dye

A9. Labeling Procedure

1. Mix 1 µL of 22 µM biotinylated MBP with 109 µL of dilution buffer to obtain 100 µL of a 200 nM MBP solution.
2. Prepare 300 µL of a 4 nM solution of the labeling dye.

Affinity of biotinylated MBP to labeling dye

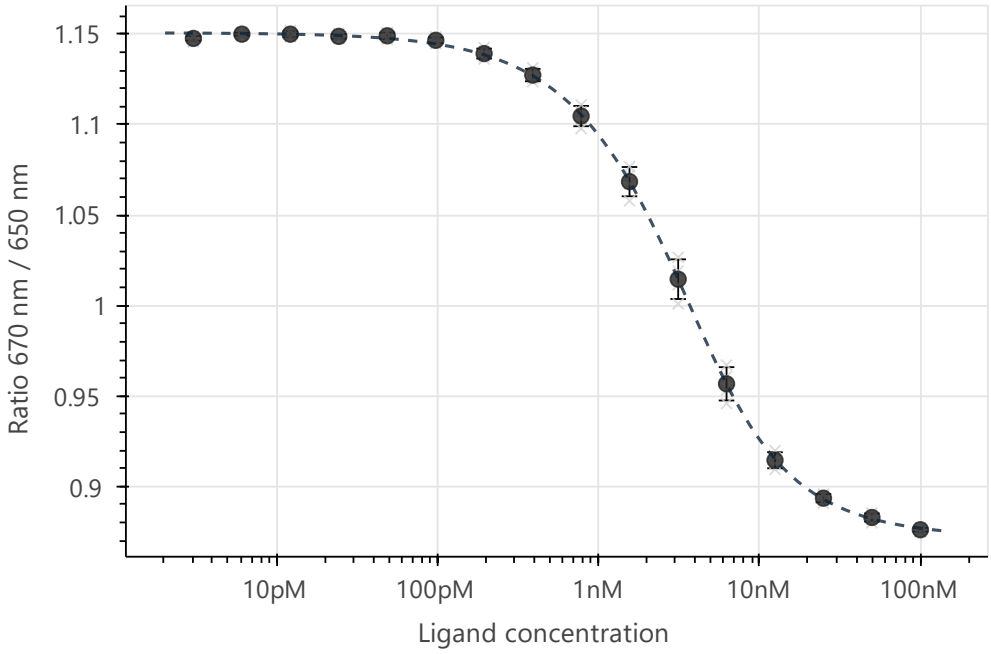
3. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 200 nM MBP solution into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
4. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **16** to get an equal volume of 10 µL for all samples.
5. Mix 100 µL of 4 nM labeling dye solution with 100 µL of dilution buffer to obtain 200 µL of a 2 nM labeling dye solution.
6. Add 10 µL of this solution to each tube from **16** to **1** and mix by pipetting.
7. Incubate for 15 minutes at room temperature in the dark before loading capillaries.

MBP labeling

8. Mix 50 µL of dilution buffer with 50 µL of 200 nM biotinylated MBP, then add 100 µL of 4 nM labeling dye solution to obtain 200 µL of a 2 nM labeling dye, 50 nM MBP solution.

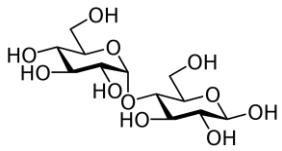
A10. Labeling Efficiency

labeling dye – biotinylated MBP | K_d = 2.35 nM (S/N = 248.0)



B1. Ligand/Non-Fluorescent Binding Partner

D-(+)-Maltose monohydrate (maltose)



B2. Molecule Class/Organism

Carbohydrate

B3. Sequence/Formula

$C_{12}H_{22}O_{11}$

B4. Purification Strategy/Source

Sigma-Aldrich GmbH
M9171

B5. Stock Concentration/Stock Buffer

72.3 mg/mL | 200 mM
ddH_2O

B6. Molecular Weight/Extinction Coefficient

360.31 Da

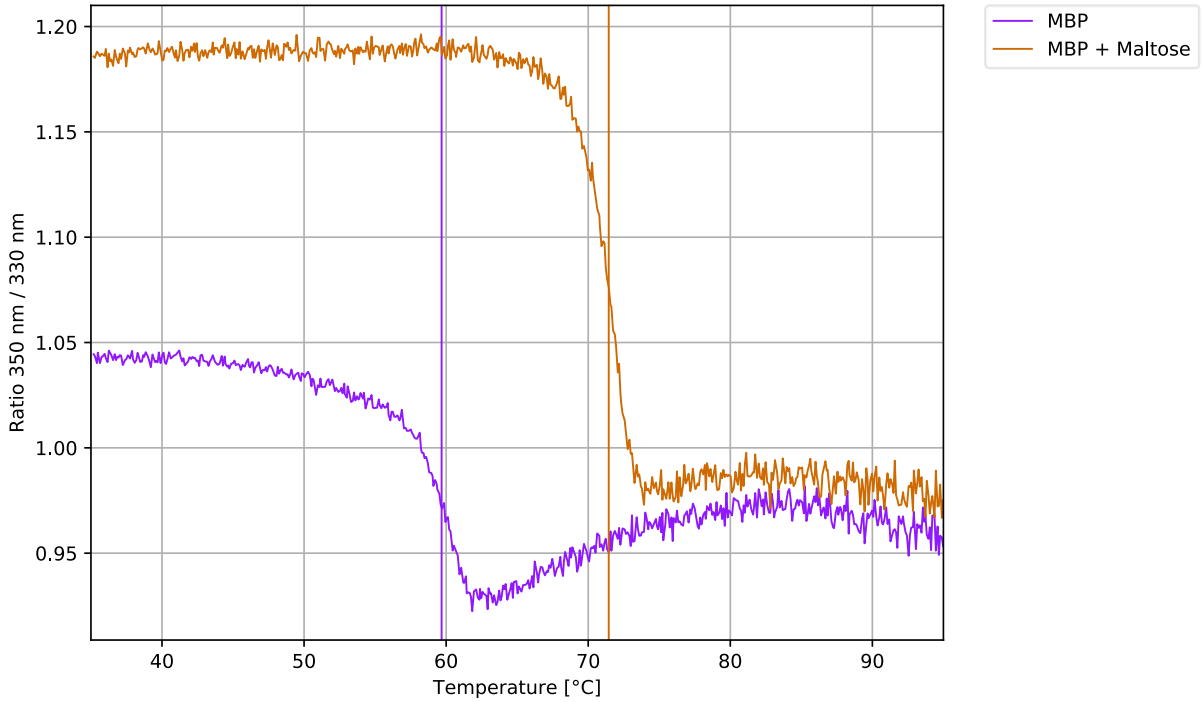
B7. Serial Dilution Preparation

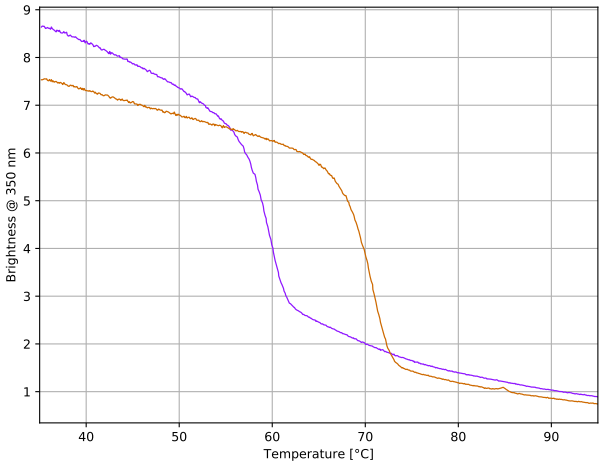
1. Dissolve 72.3 mg of maltose monohydrate in 1 mL of ddH_2O to obtain a 200 mM maltose solution.
2. Mix 2 µL of 200 mM maltose with 398 µL of dilution buffer to obtain 400 µL of a 1 mM maltose solution.
3. Take a fresh PCR tube and mix 160 µL of dilution buffer with 160 µL of 50 nM labeled MBP to obtain 320 µL of a 25 nM MBP solution.
4. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 25 nM MBP solution into tubes **2** to **16**. Then, mix 20 µL of 1 mM maltose with 20 µL of the 50 nM MBP solution in tube **1**.
5. Prepare a 1:1 serial dilution by transferring 20 µL from tube to tube. Mix carefully by pipetting up and down.
6. Incubate for 15 minutes at room temperature in the dark before loading capillaries.

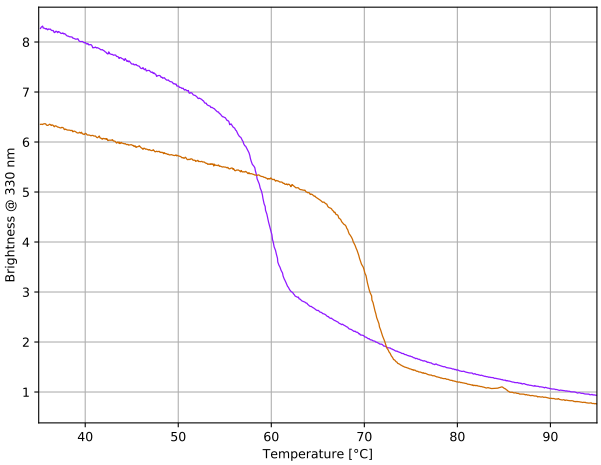
C. Tycho

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

MBP	10 µL of 200 nM MBP	$T_{i,1}$ = 59.7°C
MBP + maltose	9.5 µL of 200 nM MBP + 0.5 µL of 200 mM maltose	$T_{i,1}$ = 71.4°C







D1. Monolith System/Capillaries

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

D2. Monolith Software

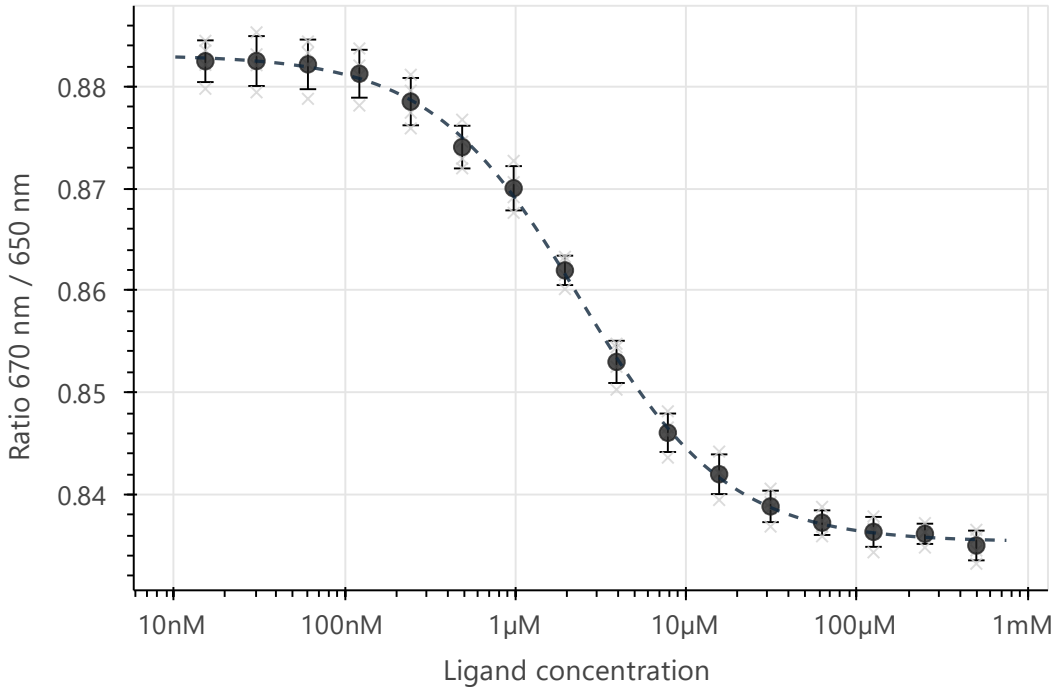
MO.Control v2.4.2 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

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

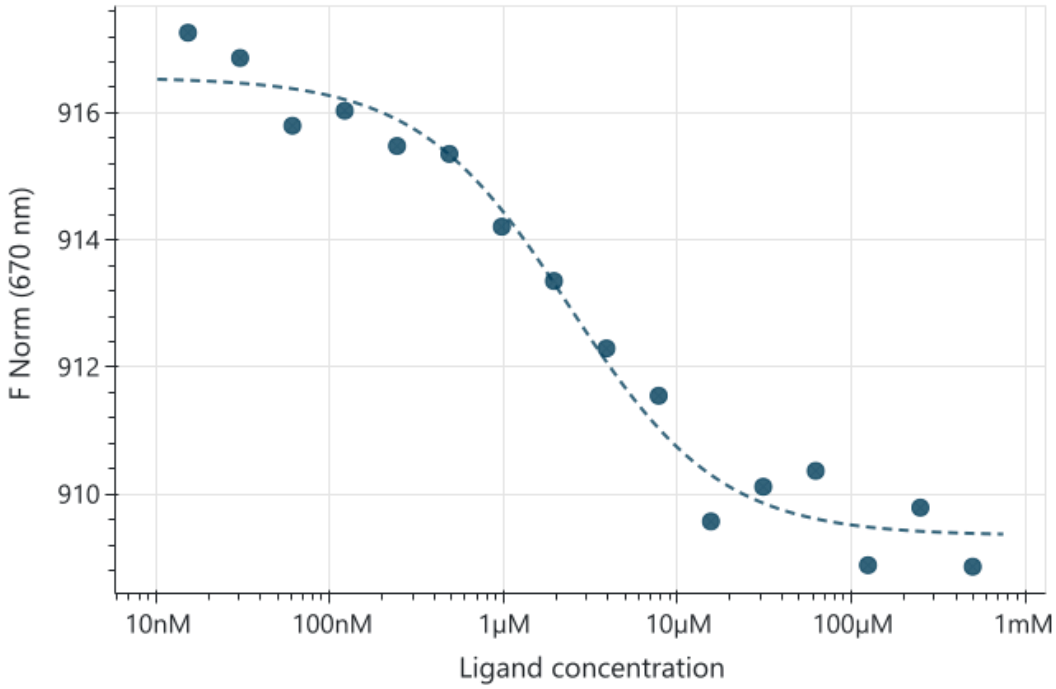
20 mM HEPES, pH 7.4, 150 mM NaCl, 0.01% Pluronic F-127®
25 nM MBP | 500 µM – 15.3 nM maltose | 25°C | 100% excitation power

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

Spectral Shift | K_d = 2.47 ± 0.07 µM (S/N = 105.9)



MST (On-Time = 1.5s) | K_d = 2.38 ± 0.52 µM (S/N = 14.6)



D5. Reference Results/Supporting Results

K_d = 830 nM Isothermal Titration Calorimetry (ITC)
Mukherjee et al., J. Biol. Chem. (2018) 293(8) 2815–2828

E. Contributors

Andreas Langer[2]

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