Monolith Protocol MO-P-007

Maltose Binding Protein – 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.

protein – small molecule interaction | carbohydrate | conformational change

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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

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KIEEGKLVIW INGDKGYNGL AEVGKKFEKD TGIKVTVEHP DKLEEKFPQV AATGDGPDII FWAHDRFGGY AQSGLLAEIT
PDKAFQDKLY PFTWDAVRYN GKLIAYPIAV EALSLIYNKD LLPNPPKTWE EIPALDKELK AKGKSALMFN LQEPYFTWPL
IAADGGYAFK YENGKYDIKD VGVDNAGAKA GLTFLVDLIK NKHMNADTDY SIAEAAFNKG ETAMTINGPW AWSNIDTSKV
NYGVTVLPTF KGQPSKPFVG VLSAGINAAS PNKELAKEFL ENYLLTDEGL EAVNKDKPLG AVALKSYEEE LAKDPRIAAT
MENAQKGEIM PNIPQMSAFW YAVRTAVINA ASGRQTVDEA LKDAQTRITK
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A4. Purification Strategy/Source

N/A

A5. Stock Concentration/Stock Buffer

0.5 mg/mL | 12 µM
Phosphate-buffered saline (PBS, pH 7.4), 10% glycerol, 0.1% Pluronic® F-127

A6. Molecular Weight/Extinction Coefficient

42 kDa
66,350 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 10 mM $MgCl_2$, 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 20 µL of a 12 µM solution MBP in PBS.
2. 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.
3. Mix 1.2 µL of the 600 µM dye solution with 18.8 µL of Labeling Buffer NHS to obtain 20 µL of a 36 µM dye solution (3x protein concentration).
4. Mix MBP and dye in a 1:1 volume ratio (40 µL final volume, 2.5% 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 40 µL of the labeling reaction from step 4 to the center of the column and let sample enter the bed completely.
9. Add 660 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. Keep the labeled MBP (~0.6 µM) on ice in the dark.

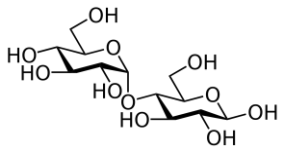
A10. Labeling Efficiency

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

Absorbance A_{280}	0.038	Protein concentration	0.52 µM
Absorbance A_{650}	0.091	Degree-of-labeling (DOL)	0.90

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

Powdered

B6. Molecular Weight/Extinction Coefficient

360.31 Da

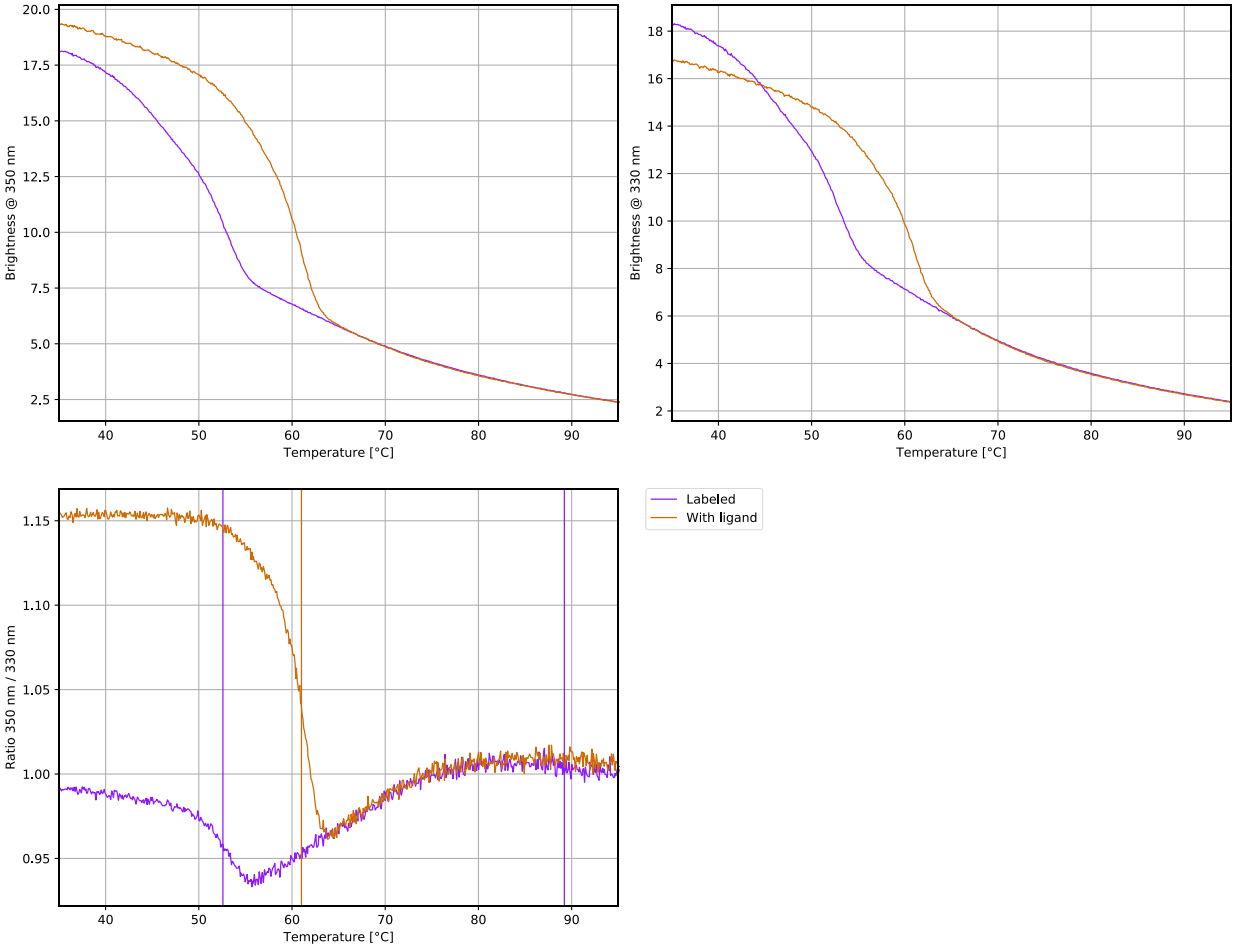
B7. Serial Dilution Preparation

1. Dissolve 10 mg of maltose monohydrate in 55.5 µL of ddH_2O to obtain a 500 mM maltose solution.
2. Mix 4 µL of 500 mM maltose with 196 µL of dilution buffer to obtain 200 µL of a 10 mM maltose solution.
3. Mix 4 µL of 10 mM maltose with 196 µL of dilution buffer to obtain 200 µL of a 200 µM maltose solution.
4. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 200 µM maltose solution into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
5. 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.
6. Mix 16 µL of labeled MBP with 184 µL of dilution buffer to obtain 200 µL of ~40 nM labeled MBP.
7. Add 10 µL of labeled MBP (~40 nM) to each tube from **16** to **1** and mix by pipetting.
8. Incubate for 5 minutes at room temperature in the dark before loading capillaries.

C. Applied Quality Checks

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

Labeled	9.5 µL of B-Column eluate (~0.6 µM) + 0.5 µL of dilution buffer	T_i = 52.6°C
With ligand	9.5 µL of B-Column eluate (~0.6 µM) + 0.5 µL of 10 mM maltose	T_i = 61.0°C



D1. MST System/Capillaries

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

D2. MST Software

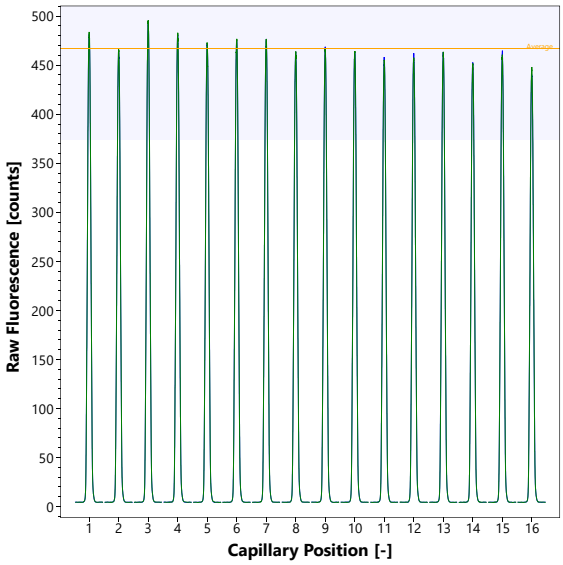
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

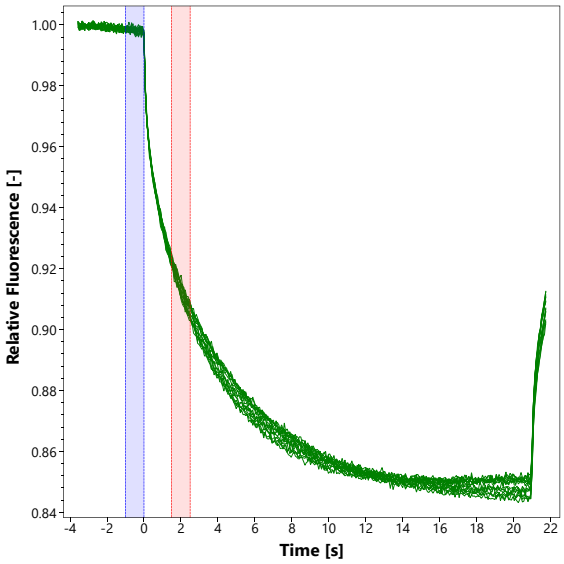
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

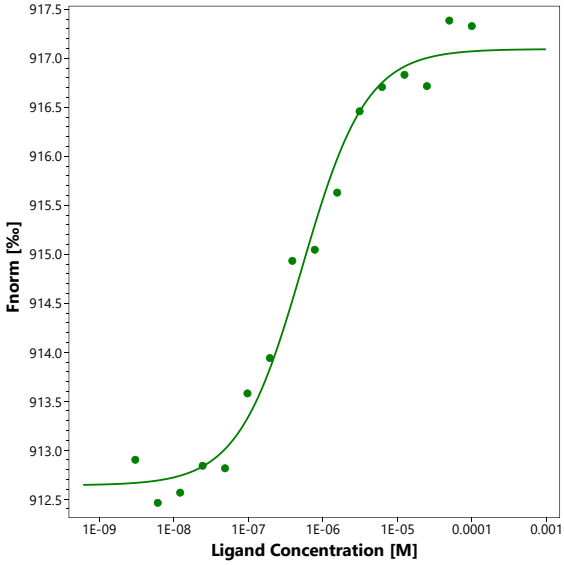
50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 10 mM $MgCl_2$, 0.05% TWEEN® 20
20 nM MBP | 100 µM – 3 nM maltose | 25°C | medium MST power | 40% excitation power

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

K_d = 520 nM







D5. Reference Results/Supporting Results

K_d = 1.2 µM Intrinsic fluorescence changes
Telmer and Shilton, J Biol Chem 278 (2003) 34555-34567

E. Contributors

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