Monolith Protocol MO-P-008

Maltose Binding Protein – Maltose (label-free)

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 | label-free

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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
```

A4. Purification Strategy/Source

N/A

A5. Stock Concentration/Stock Buffer

0.5 mg/mL | 12 µM
Phosphate-buffered saline, 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

Phosphate-buffered saline (PBS, pH 7.4)

A8. Labeling Strategy

Trp and Tyr fluorescence

A9. Labeling Procedure

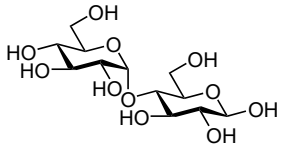
1. Mix 4 µL of Pluronic® F-127 (5%) with 187 µL of PBS.
2. Add 8.3 µL of 12 µM MBP to obtain 200 µL of a 500 nM MBP solution in PBS with 0.1% Pluronic® F-127.

A10. Labeling Efficiency

N/A

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

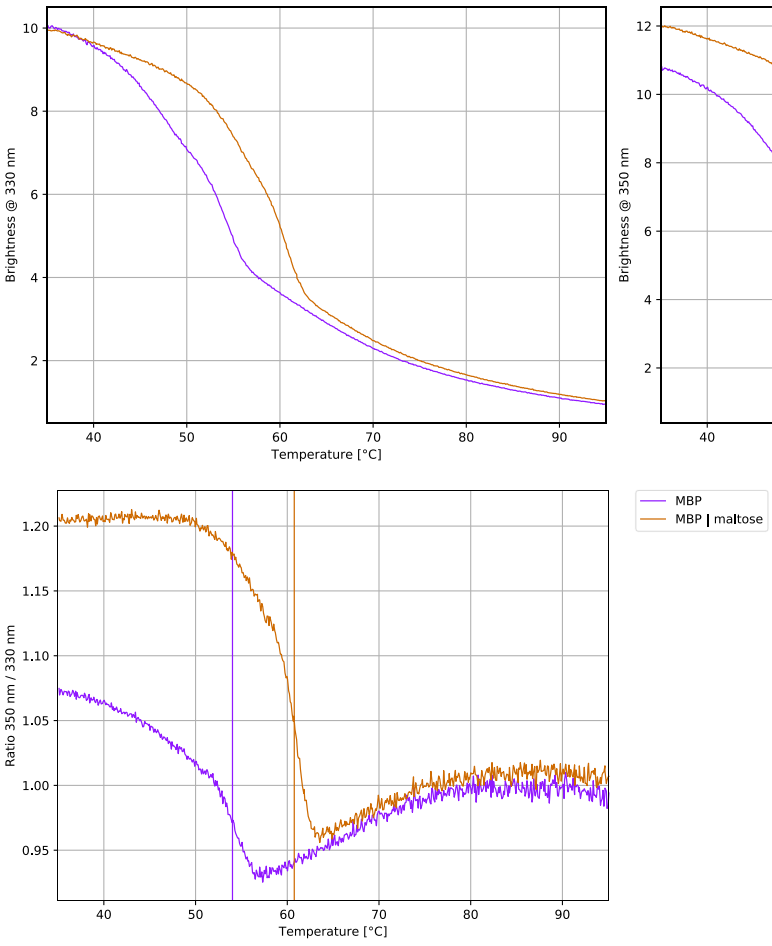
B7. Serial Dilution Preparation

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 8 µL of 10 mM maltose with 192 µL of dilution buffer to obtain 200 µL of a 400 µM maltose solution.
4. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 400 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. Add 10 µL of 500 nM MBP to each tube from **16** to **1** and mix by pipetting.
7. Incubate for 5 minutes at room temperature in the dark before loading capillaries.

C. Applied Quality Checks

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

MBP	5 µL of 500 nM MBP + 5 µL of dilution buffer (tube **16**)	T_i = 54.0°C
MBP | maltose	5 µL of 500 nM MBP + 5 µL of 400 µM maltose (tube **1**)	T_i = 60.8°C



D1. MST System/Capillaries

Monolith NT.LabelFree (NanoTemper Technologies GmbH)
Capillaries Monolith NT.LabelFree (MO-Z022, NanoTemper Technologies GmbH)

D2. MST Software

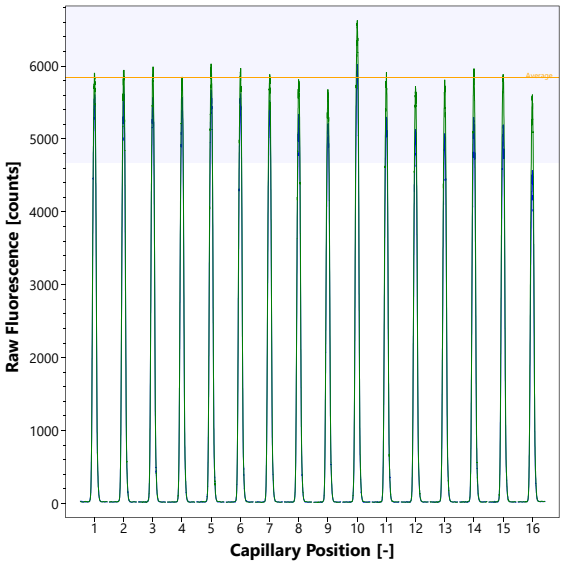
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

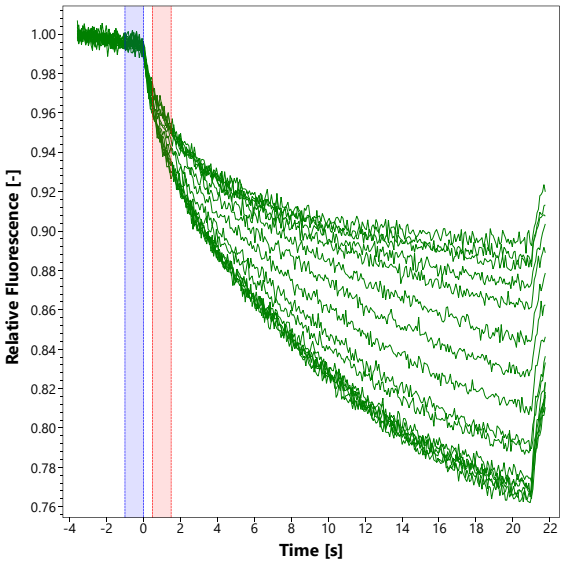
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

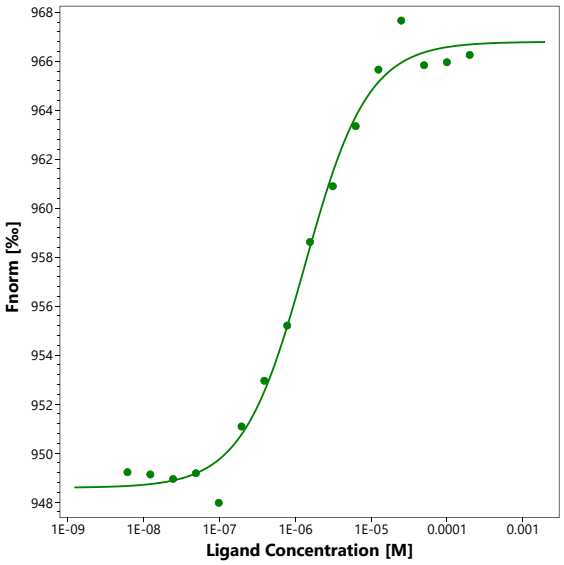
Phosphate-buffered saline (PBS, pH 7.4), 0.05% Pluronic® F-127
250 nM MBP | 200 µM – 6.1 nM maltose | 25°C | medium MST power | 10% excitation power

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

K_d = 1.22 µM







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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