Monolith X Protocol MOX-P-107

TEM-116 – BLIP

Beta-Lactamase Inhibitor Protein (BLIP) is a secreted protein from *Streptomyces clavuligerus* that inhibits a wide range of beta-lactamases. TEM-116 is a broad-spectrum beta-lactamase found in many species of bacteria.

protein – protein interaction | beta-lactamase

A1. Target/Fluorescent Molecule

TEM-116
uniprot.org/uniprot/A0A0F6T744

A2. Molecule Class/Organism

Beta-lactamase
Escherichia coli

A3. Sequence/Formula

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MMSIQHFRVA LIPFFAAFCL PVFAHPETLV KVKDAEDQLG ARVGYIELDL NSGKILESFR PEERFPMMST FKVLLCGAVL
SRIDAGQEQL GRRIHYSQND LVEYSPVTEK HLTDGMTVRE LCSAAITMSD NTAANLLLTT IGGPKELTAF LHNMGDHVTR
LDRWEPELNE AIPNDERDTT MPVAMATTLR KLLTGELLTL ASRQQLIDWM EADKVAGPLL RSALPAGWFI ADKSGAGERG
SRGIIAALGP DGKPSRIVVI YTTGSQATMD ERNRQIAEIG ASLIKHW
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A4. Purification Strategy/Source

Expressed in *E.coli* BL21 (DE3)
Crelux GmbH

A5. Stock Concentration/Stock Buffer

2.59 mg/mL | 81.7 µM
50 mM Tris-HCl, pH 8.4, 100 mM NaCl

A6. Molecular Weight/Extinction Coefficient

31.7 kDa
28,085 $M^{-1}cm^{-1}$ (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* A-Column | 1* B-Column

A9. Labeling Procedure

1. Add 35 µL of Labeling Buffer NHS to 5 µL of 81.7 µM TEM-116 to obtain 40 µL of a 10 µM solution.
2. Use the A-Column to perform a buffer exchange into Labeling Buffer NHS.
   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 NHS and centrifuge at **1500 × g** for **1 min** (3x).
   e. Place 40 µL of the 10 µM TEM-116 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 40 µL of ~7.5 µM TEM-116 (70 – 80% recovery).
3. 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.
4. Mix 2 µL of the 600 µM dye solution with 38 µL of Labeling Buffer NHS to obtain 40 µL of a 30 µM dye solution (4x protein concentration).
5. Mix TEM-116 and dye in a 1:1 volume ratio (80 µL final volume, 2.5% final DMSO concentration).
6. Incubate for 30 minutes at room temperature in the dark.
7. 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.
8. 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.
9. Add 80 µL of the labeling reaction from step 5 to the center of the column and let sample enter the bed completely.
10. Add 520 µL of dilution buffer after the sample has entered and discard the flow through.
11. Place column in a new collection tube, add 500 µL of dilution buffer and collect the eluate.
12. 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.
13. Prepare 10 µL aliquots of the labeled TEM (~0.6 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_{205}	0.420	Protein concentration	0.41 µM
Absorbance A_{650}	0.070	Degree-of-labeling (DOL)	0.87

B1. Ligand/Non-Fluorescent Binding Partner

BLIP
uniprot.org/uniprot/P35804

B2. Molecule Class/Organism

Beta-lactamase inhibitory protein
Streptomyces clavuligerus

B3. Sequence/Formula

MAGVMGAKFT QIQFGMTRQQ VLDIAGAENC ETGGSFGDSI HCRGHAAGDY YAYATFGFTS AAADAKVDSK SQEKLLAPSA PTLTLAKFNQ VTVGMTRAQV LATVGQGSCT TWSEYYPAYP STAGVTLSLS CFDVDGYSST GFYRGSAHLW FTDGVLQGKR QWDLV

B4. Purification Strategy/Source

Expressed in *E.coli* BL21 (DE3)
Crelux GmbH

B5. Stock Concentration/Stock Buffer

1.2 mg/mL | 68 µM
50 mM Tris-HCl, pH 8.4, 500 mM NaCl

B6. Molecular Weight/Extinction Coefficient

17.7 kDa
28,670 $M^{-1}cm^{-1}$ (ε_{280})

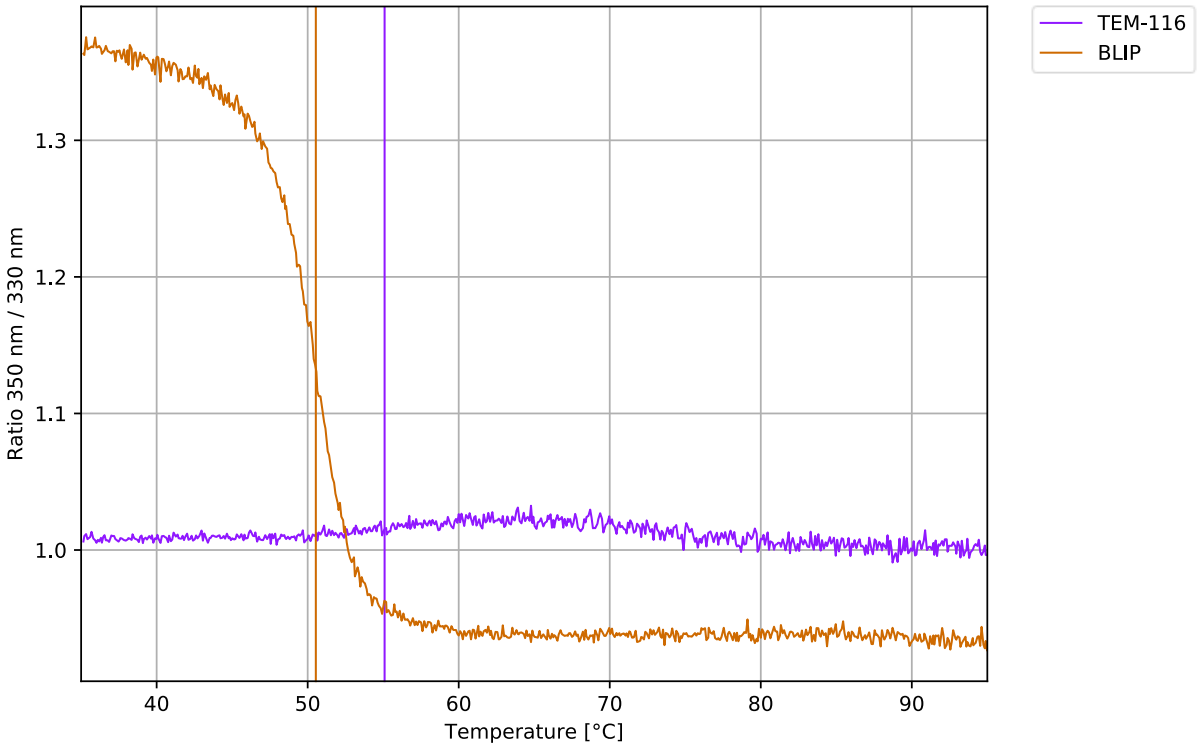
B7. Serial Dilution Preparation

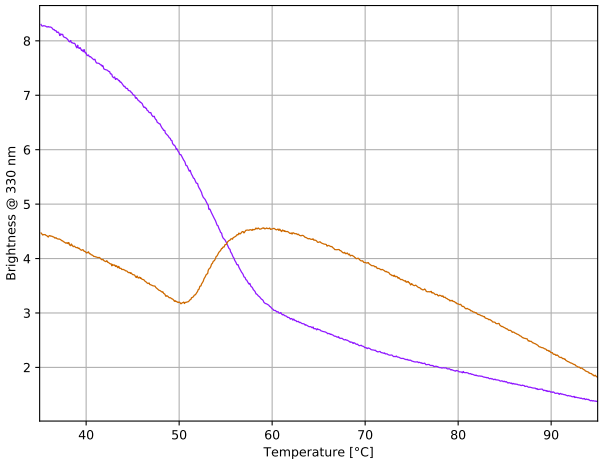
1. Add 2 µL of the 68 µM BLIP solution to 66 µl of dilution buffer to obtain 68 µL of a 2 µM BLIP solution.
2. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 2 µM BLIP solution into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
3. Prepare a 1:1 serial dilution 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 13.3 µL of labeled TEM-116 with 186.7 µL of dilution buffer to obtain 200 µL of ~40 nM TEM-116.
5. Add 10 µL of labeled TEM-116 (~40 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.

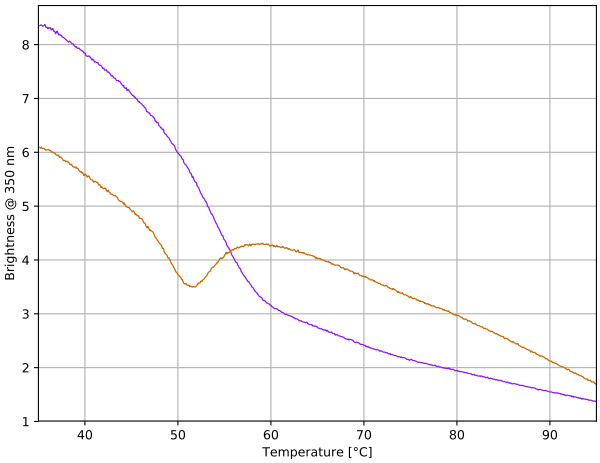
C. Tycho

Validation of structural integrity of TEM-116 and BLIP using Tycho NT.6:
nanotempertech.com/tycho

TEM-116	10 µL of B-Column eluate (~0.6 µM)	T_i = 55.1°C
BLIP	10 µL of 2 µM BLIP solution	T_i = 50.5°C







D1. Monolith System/Capillaries

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

D2. Monolith Software

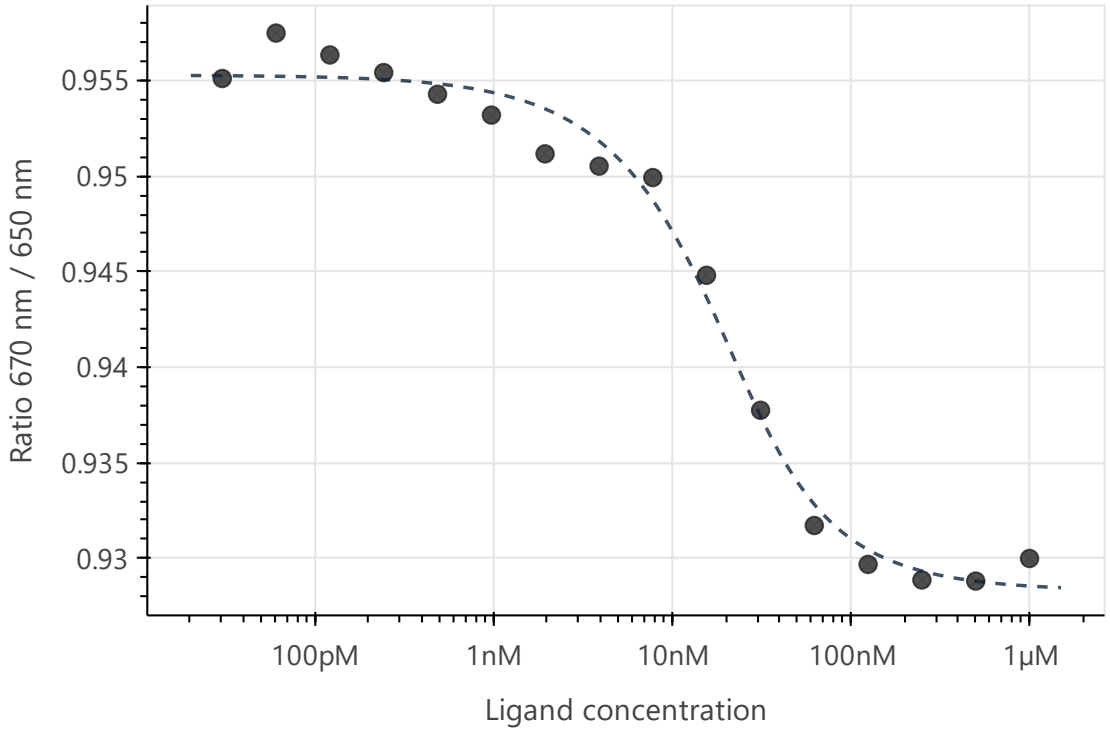
MO.Control v2.4.2 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

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

50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 10 mM $MgCl_2$, 0.05% TWEEN® 20
20 nM TEM | 1 µM – 30.5 pM BLIP | 25°C | 40% excitation power

D4. Monolith Results (Dose Response)

K_d = 9.23 ± 2.2 nM (S/N = 21.8)



D5. Reference Results/Supporting Results

N/A

E. Contributors

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