Monolith Protocol MO-P-001

p38-alpha – SB 203580

Mitogen-activated protein kinase 14 (also called p38-α) is an enzyme that has been implicated in the regulation of many proinflammatory pathways. It is a drug target for many diseases such as rheumatoid arthritis, endotoxic shock and osteoporosis. SB 203580 is a specific and potent inhibitor of kinases that binds p38-α with nM affinity.

protein – small molecule | kinase | inhibitor | His_6-tag

A1. Target/Fluorescent Molecule

Mitogen-activated protein kinase 14 (p38-α)
uniprot.org/uniprot/Q16539

A2. Molecule Class/Organism

p38 mitogen-activated protein kinase (MAP kinase)
Homo sapiens (Human)

A3. Sequence/Formula

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MSQERPTFYR QELNKTIWEV PERYQNLSPV GSGAYGSVCA AFDTKTGLRV AVKKLSRPFQ SIIHAKRTYR ELRLLKHMKH
ENVIGLLDVF TPARSLEEFN DVYLVTHLMG ADLNNIVKCQ KLTDDHVQFL IYQILRGLKY IHSADIIHRD LKPSNLAVNE
DCELKILDFG LARHTDDEMT GYVATRWYRA PEIMLNWMHY NQTVDIWSVG CIMAELLTGR TLFPGTDHID QLKLILRLVG
TPGAELLKKI SSESARNYIQ SLTQMPKMNF ANVFIGANPL AVDLLEKMLV LDSDKRITAA QALAHAYFAQ YHDPDDEPVA
DPYDQSFESR DLLIDEWKSL TYDEVISFVP PPLDQEEMES
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A4. Purification Strategy/Source

Expressed in E. coli BL21, His_6-tagged
Crelux GmbH

A5. Stock Concentration/Stock Buffer

8.82 mg/mL | 197 µM
25 mM HEPES, pH 7.4, 50 mM NaCl, 10 mM DTT, 1 mM EDTA

A6. Molecular Weight/Extinction Coefficient

44.7 kDa
50,100 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

20 mM HEPES, pH 7.4, 150 mM NaCl, 0.005% TWEEN® 20

A8. Labeling Strategy

Monolith His-Tag Labeling Kit RED-tris-NTA 2nd Generation (MO-L018, NanoTemper Technologies GmbH)
1* 125 pmol RED-tris-NTA Dye 2nd Generation

A9. Labeling Procedure

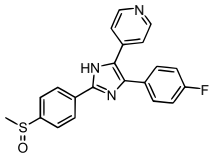
1. Add 195 µL of dilution buffer to 2 µL of 197 µM p38-α to obtain 197 µL of a 2 µM solution.
2. Suspend 125 pmol RED-tris-NTA Dye 2nd Generation in 25 µL of dilution buffer to obtain a 5 µM dye solution.
3. Mix 188 µL of dilution buffer with 2 µL dye (5 µM) and 10 µL p38-α (2 µM) to obtain 200 µL of a 100 nM p38-α, 50 nM dye solution.
4. Incubate for 30 minutes at room temperature in the dark.

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

SB 203580



B2. Molecule Class/Organism

p38 MAPK inhibitor

B3. Sequence/Formula

$C_{21}H_{16}FN_3OS$

B4. Purification Strategy/Source

Sigma-Aldrich GmbH
S8307

B5. Stock Concentration/Stock Buffer

1 mg/mL | 2.65 mM
DMSO

B6. Molecular Weight/Extinction Coefficient

377.43 Da

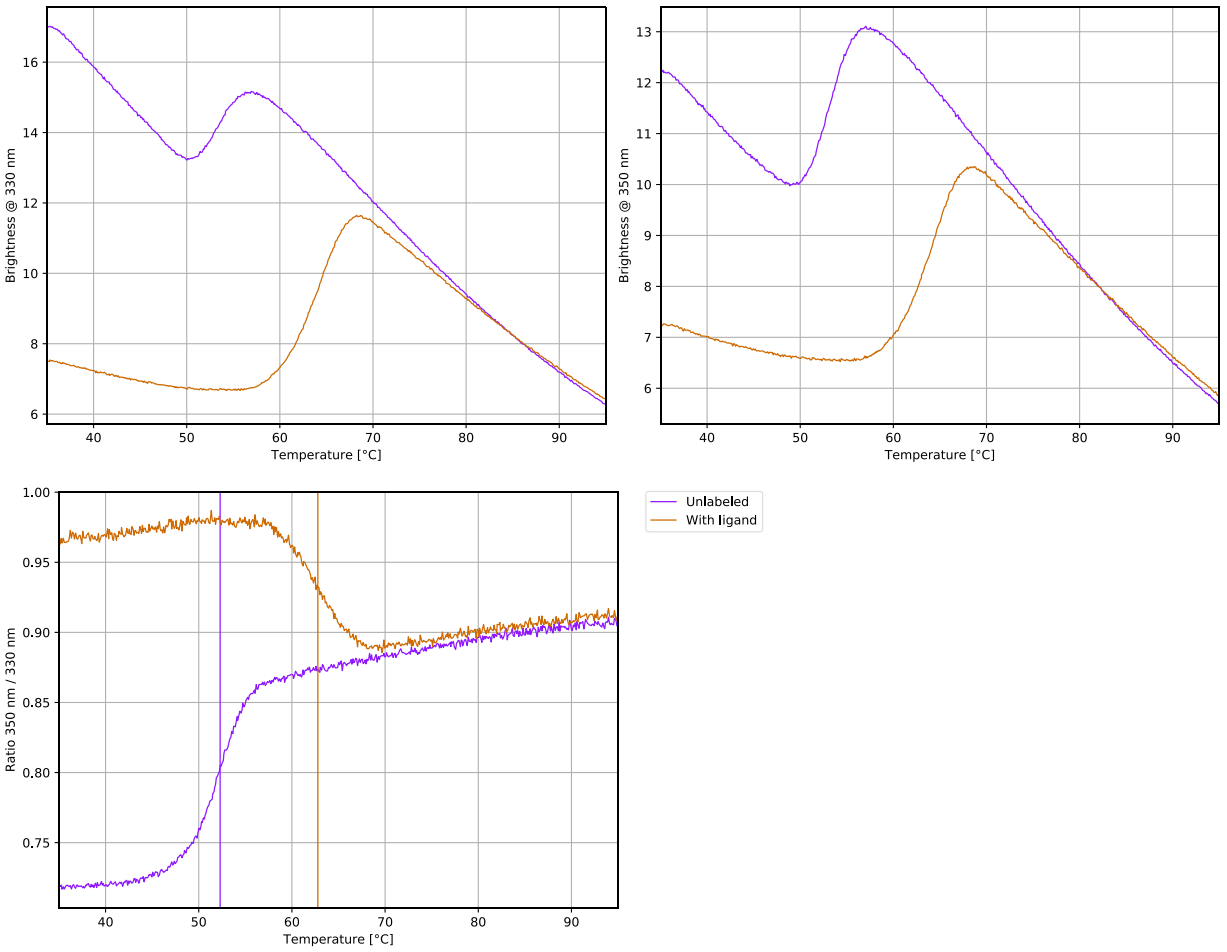
B7. Serial Dilution Preparation

1. Add 8.6 µL of DMSO to 2 µL of the SB 203580 stock to obtain 10.6 µL of a 500 µM solution.
2. Mix 2 µL of the 500 µM SB 203580 solution with 98 µL of dilution buffer to obtain 100 µL of a 10 µM SB 203580 solution.
3. Mix 4 µL of DMSO with 196 µL of dilution buffer to obtain 200 µL of a 2% DMSO solution.
4. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 20 µM SB 203580 solution into tube **1**. Then, transfer 10 µL of the 2% DMSO solution 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 labeled p38-α (100 nM) to each tube from **16** to **1** and mix by pipetting.
7. Incubate for 20 minutes at room temperature in the dark before loading capillaries.

C. Applied Quality Checks

Validation of structural integrity and functionality of labeled p38-α using Tycho NT.6:
nanotempertech.com/tycho

Unlabeled	5 µL of 2 µM p38-α + 5 µL of dilution buffer containing 2% DMSO	T_i = 52.3°C
With ligand	5 µL of 2 µM p38-α + 5 µL of 10 µM SB 203580	T_i = 62.8°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

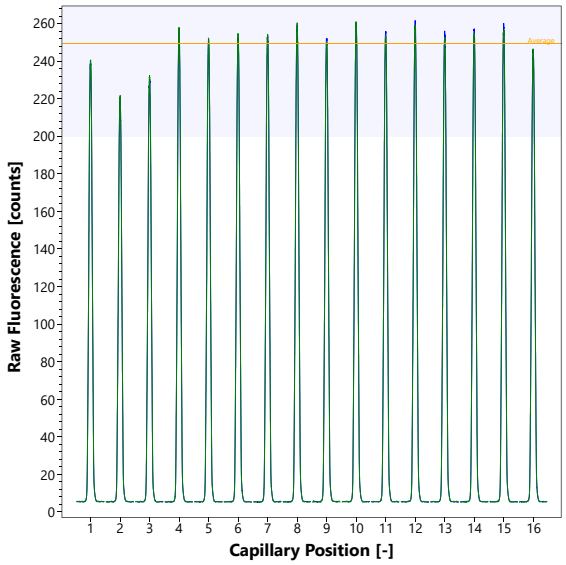
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

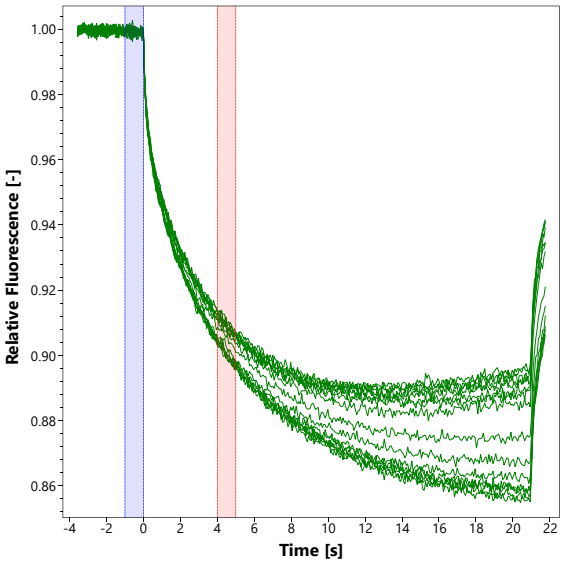
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

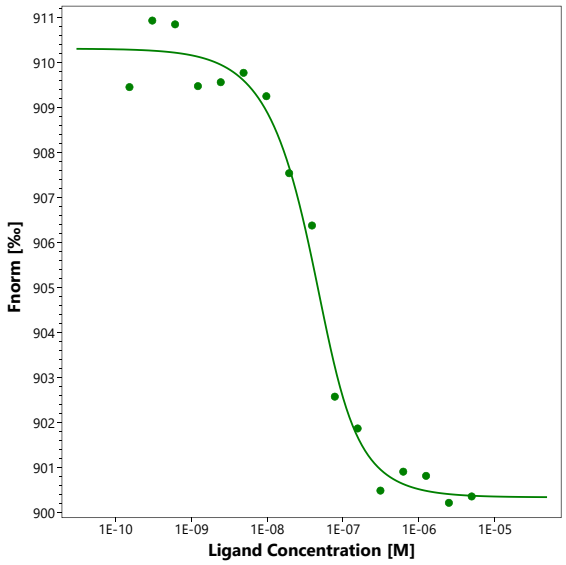
20 mM HEPES, pH 7.4, 150 mM NaCl, 0.005% TWEEN® 20, 1% DMSO
50 nM p38-α | 5 µM – 153 pM SB 203580 | 25°C | medium MST power | 50% excitation power

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

K_d = 17.5 nM







D5. Reference Results/Supporting Results

K_d = 21 nM	Surface Plasmon Resonance (SPR) Thurmond et al., Eur J Biochem 268 (2001) 5747–5754
K_d = 15 nM	Isothermal Titration Calorimetry (ITC) Young et al., J Biol Chem 272 (1997) 12116-12121

E. Contributors

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