Monolith X Protocol MOX-P-118

KRAS G12D (biotinylated) – BI-2852 & MRTX1133

BI-2852 is a potent inhibitor for in vitro use that directly targets GTP-bound KRAS, which is the major form present in cancer cells carrying KRAS mutations. MRTX1133 is a noncovalent, potent, and selective KRAS G12D inhibitor which optimally fills the switch II pocket and extends three substituents to favorably interact with the protein. It selectively inhibits KRAS G12D mutant, but not KRAS wild-type, tumor cells. MRTX1133 has single digit nanomolar activity in cellular assays and marked in vivo efficacy in tumor models harboring KRAS G12D mutations.

protein – small molecule | biotinylated target | competition assay | thermodynamics | van't Hoff analysis | enthalpy | entropy

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A1. Target/Fluorescent Molecule

$KRAS^{G12D}$
uniprot.org/uniprot/P01116

A2. Molecule Class/Organism

GTPase
Human (Homo sapiens)

A3. Sequence/Formula

MTEYKLVVVG ADGVGKSALT IQLIQNHFVD EYDPTIEDSY RKQVVIDGET CLLDILDTAG QEEYSAMRDQ YMRTGEGFLC
VFAINNTKSF EDIHHYREQI KRVKDSEDVP MVLVGNKCDL PSRTVDTKQA QDLARSYGIP FIETSAKTRQ GVDDAFYTLV
REIRKHKEK

A4. Purification Strategy/Source

N-terminal His-tag and Avi-tag, biotinylated
Sino Biological
12259-H56E-B

A5. Stock Concentration/Stock Buffer

0.25 mg/mL | 10.5 µM
50 mM Tris-HCl, pH 8.0

A6. Molecular Weight/Extinction Coefficient

23.8 kDa
13,410 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

20 mM HEPES, pH 7.4, 150 mM NaCl, 2 mM $MgCl_2$, 0.5 mM TCEP, 0.01 % TWEEN® 20[1]

A8. Labeling Strategy

Biotinylated Target Labeling Kit (Cat# NT-L020)
1* 20 pmol labeling dye (50 µL, 400 nM)

A9. Labeling Procedure

1. Mix 1.9 µL of 10.5 µM KRAS with 18.1 µL of dilution buffer to obtain 20 µL of a 1 µM KRAS solution.
2. Mix 1 µL of labeling dye with 99 µL of dilution buffer to obtain 100 µL of a 4 nM dye solution.

Affinity of dye to KRAS

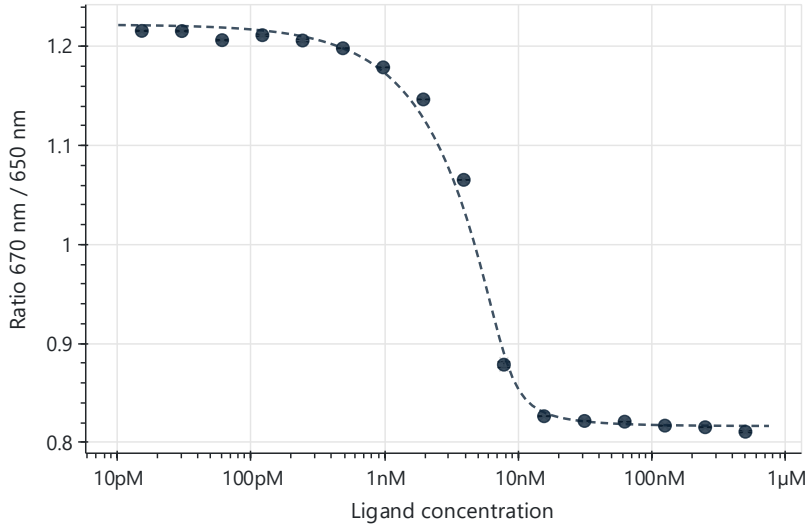
3. Take a PCR tube and mix 80 µL of 4 nM dye with 80 µL of dilution buffer to obtain 160 µL of a 2 nM dye solution.
4. Prepare a PCR-rack with 16 PCR tubes. Transfer 10 µL of the 2 nM dye solution into tubes **2** to **16**. Then, mix 10 µL of 1 µM KRAS with 10 µL of 4 nM dye in tube **1**.
5. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down.
6. Incubate for 30 minutes at room temperature in the dark before loading capillaries.

KRAS labeling

7. Mix 4 µL of 400 nM dye with 0.8 µL of KRAS and 195.2 µL of dilution buffer to obtain 200 µL of a 40 nM KRAS, 8 nM dye solution. Keep labeled KRAS on ice in the dark.

A10. Labeling Efficiency

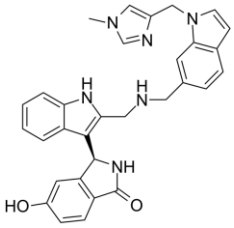
Affinity between labeling dye and biotinylated KRAS – K_d = 277 ± 107 pM (S/N = 38.2)



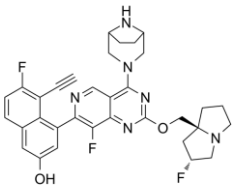
[1] To prepare the dilution buffer, mix 100 µL of HEPES (1 M, pH 7.4) with 250 µL of NaCl (3 M), 5 µL of TCEP (500 mM), 10 µL of $MgCl_2$ (1 M), 5 µL of TWEEN® 20 (10%) and 4.6 mL of ddH_2O.

B1. Ligand/Non-Fluorescent Binding Partner

BI-2852



MRTX1133



B2. Molecule Class/Organism

KRAS inhibitors

B3. Sequence/Formula

$C_{31}H_{28}N_6O_2$

$C_{33}H_{31}F_3N_6O_2$

B4. Purification Strategy/Source

opnme.com
BI-2852

MedChemExpress
HY-134813

B5. Stock Concentration/Stock Buffer

1 mg/mL | 2 mM
DMSO

1.2 mg/mL | 2 mM
DMSO

B6. Molecular Weight/Extinction Coefficient

516.59 Da

600.63 Da

B7. Serial Dilution Preparation

BI-2852

1. Prepare a 2 mM stock solution of BI-2852 in DMSO.
2. Mix 2 µL of 2 mM BI-2852 with 48 µL of dilution buffer to obtain 48 µL of an 80 µM BI-2852 solution.
3. Mix 20 µL of DMSO with 480 µL of dilution buffer to obtain 500 µL of dilution buffer with 4% DMSO.
4. Take a PCR tube and mix 40 µL of dilution buffer with 40 µL of 40 nM KRAS, 8 nM dye to obtain 80 µL of 20 nM KRAS, 4 nM dye.
5. Take a fresh PCR tube and mix 60 µL of dilution buffer containing 4% DMSO with 60 µL of 20 nM KRAS, 4 nM dye to obtain 120 µL of a 10 nM KRAS, 2 nM dye solution.
6. Prepare a PCR-rack with 12 PCR tubes. Transfer 10 µL of the 10 nM KRAS, 2 nM dye solution into tubes **2** to **12**. Then, mix 10 µL of 80 µM BI-2852 with 10 µL of 20 nM KRAS, 4 nM dye in tube **1**.
7. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down.
8. Incubate for 5 minutes at room temperature in the dark before loading capillaries.

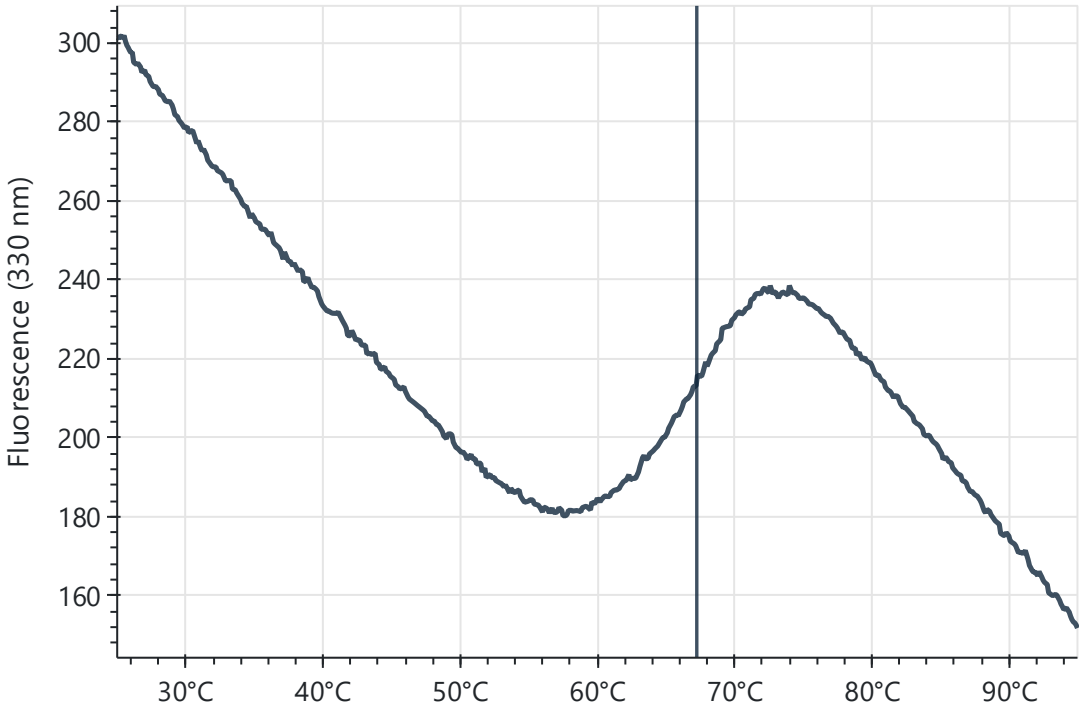
MRTX1133 (competitive assay)

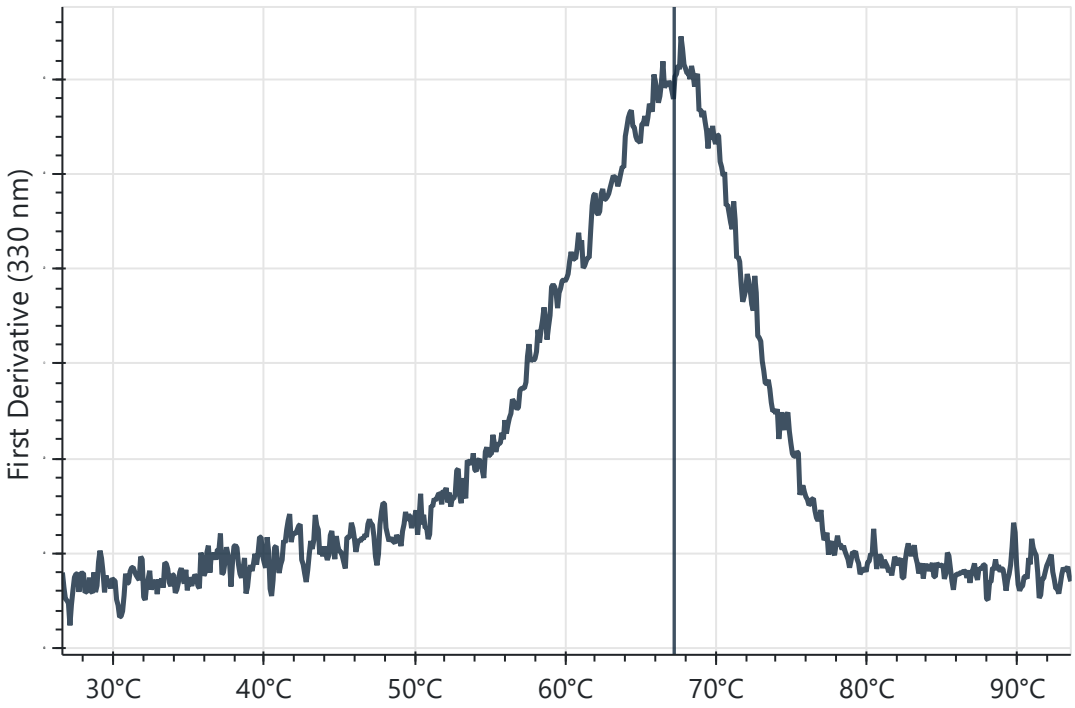
1. Prepare a 2 mM stock solution of MRTX1133 in DMSO.
2. Mix 1 µL of 2 mM MRTX1133 with 999 µL of dilution buffer to obtain 1 mL of 2 µM MRTX1133.
3. Mix 4 µL of 2 mM BI-2852 with 46 µL of dilution buffer. Then, add 50 µL of 40 nM KRAS, 8 nM dye to obtain 100 µL of a 20 nM KRAS, 4 nM dye, 80 µM BI-2852 solution.
4. Take a fresh PCR tube and mix 80 µL of dilution buffer with 80 µL of 20 nM KRAS, 4 nM dye, 80 µM BI-2852 to obtain 160 µL of a 10 nM KRAS, 2 nM dye, 40 µM BI-2852 solution.
5. Prepare a PCR-rack with 16 PCR tubes. Transfer 10 µL of the 10 nM KRAS, 2 nM dye, 40 µM BI-2852 solution into tubes **2** to **16**. Then, mix 10 µL of 2 µM MRTX1133 with 10 µL of 20 nM KRAS, 4 nM dye, 80 µM BI-2852 in tube **1**.
6. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down.
7. Incubate for 15 minutes at room temperature in the dark before loading capillaries.

C. Prometheus Panta

Validation of structural integrity of KRAS using Prometheus Panta (heating ramp of 7°C/min):
nanotempertech.com/prometheus

KRAS	10 µL of 1 µM KRAS	T_i = 67.25°C





D1. Monolith System/Capillaries

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

D2. Monolith Software

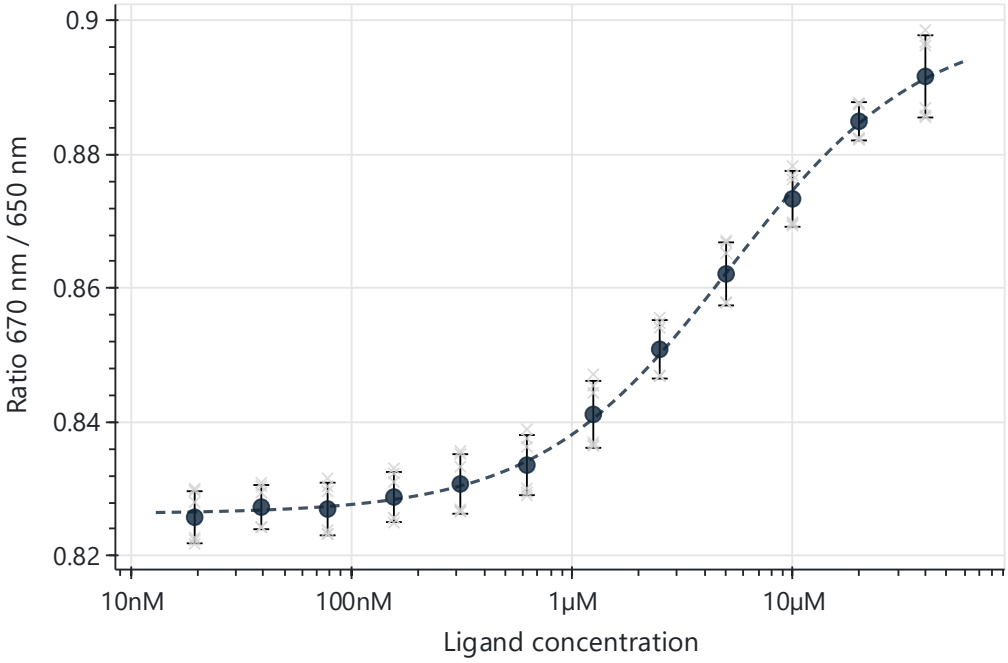
MO.Control v2.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

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

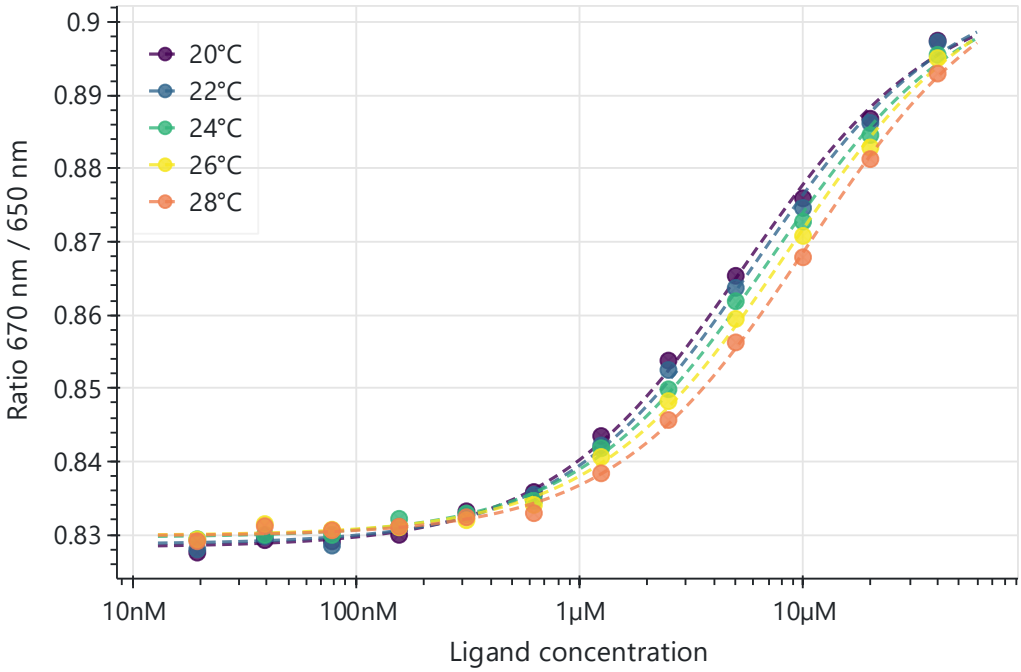
20 mM HEPES, pH 7.4, 150 mM NaCl, 2 mM $MgCl_2$, 0.5 mM TCEP, 0.01 % TWEEN® 20, 2% DMSO
10 nM $KRAS^{G12D}$ (2 nM dye) | 40 µM –19.5 nM BI-2852 | 20°C | 100% Excitation Power

D4. Monolith Results (Dose Response)

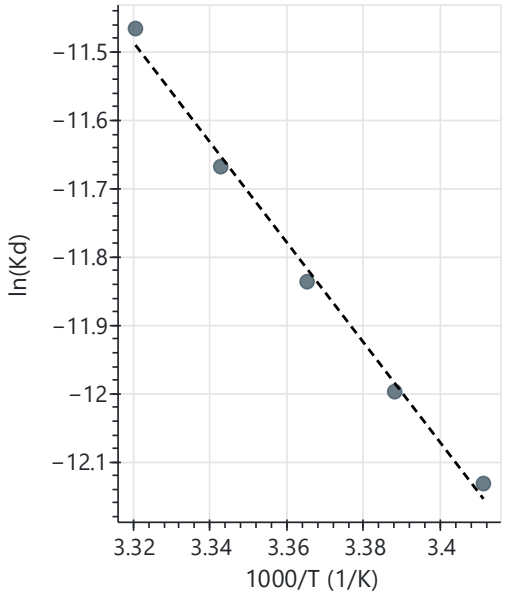
$KRAS^{G12D}$ – BI-2852 | K_d = 5.21 ± 0.22 µM (S/N = 103.3)

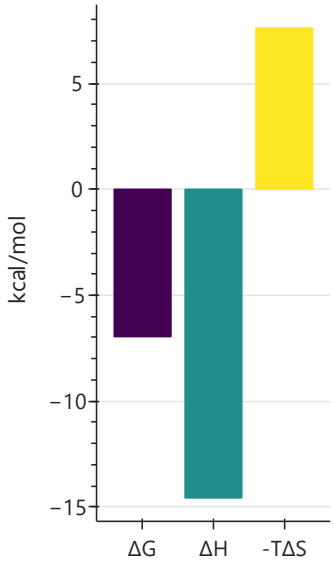


Temperature dependence of BI-2852 binding to KRAS^{G12D}



Van't Hoff analysis[2]



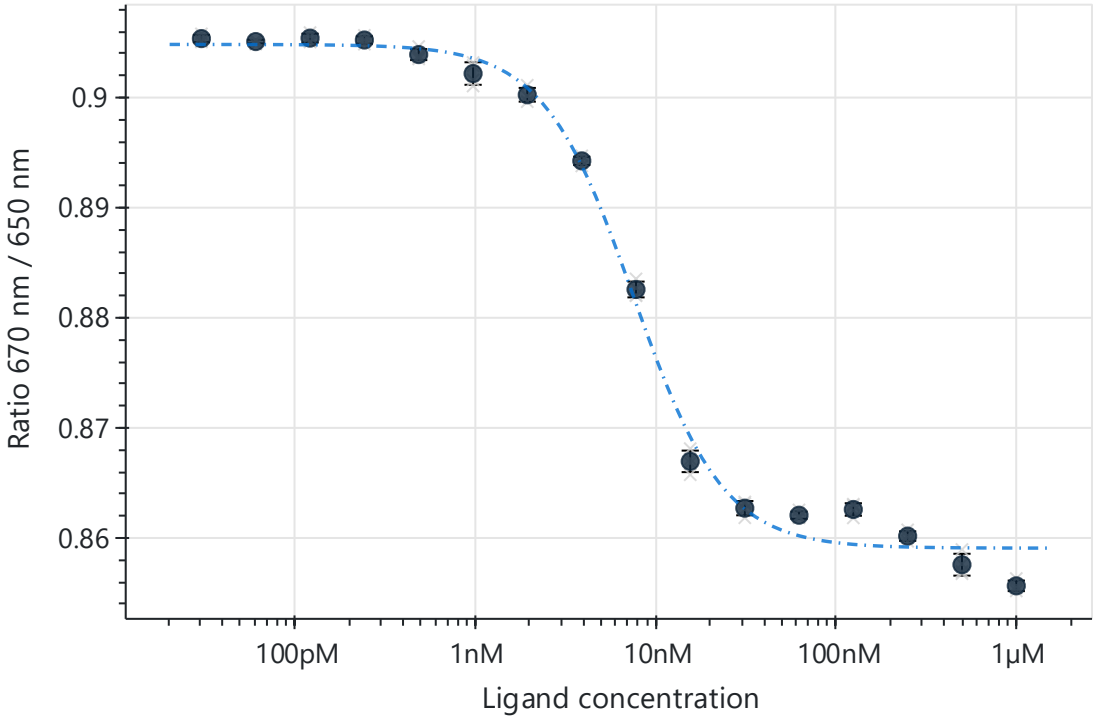


ΔH = -14.6 ± 0.7 kcal/mol

ΔS = -25.5 ± 2.3 cal/mol/K

[2] Calculations can be performed with Monolith X's Thermodynamics Measurement mode in MO.Control 2.7.0 and later versions. Plots were created outside of MO.Control 2 with temperature set at 25°C (298.15K) for ΔG calculation.

KRAS^{G12D} (in complex with 40 µM BI-2852) – MRTX1133 | EC_{50} = 7.56 ± 0.55 nM (S/N = 28.4) | K_i = 0.3 nM[3]



D5. Reference Results/Supporting Results

BI-2852	K_D = 2.0 µM (GDP KRAS^{G12D})	Isothermal Titration Calorimetry (ITC) Kessler et al, PNAS 116, 15823-15829 (2019)
MRTX1133	K_D = 0.2 pM	Surface Plasmon Resonance (SPR) Wang et al, J. Med. Chem. 2022, 65, 3123−3133 (2022)

E. Contributors

Andreas Langer[4]

[3] Calculated with Nanotemper Technologies' K_I calculator (input: 40.000 nM BI-2852 for *2. concentration of fluorescent competitor* and 10 nM labelled KRAS^{G12D} for *3. concentration of unlabeled target* | nanotempertech.com/user-tools/ki-calculator

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