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Zika virus NS2B-NS3 protease binding assay for compound screen using grating-coupled interferometry

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

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Abstract

This protocol aims to measure kinetic parameters (ka, kd, and KD) for compounds against Zika virus NS2B-NS3 protease using the Creoptix Wave system. Grating coupled interferometry (GCI) is a label-free technique to measure kinetics and affinity for different targets (proteins, small molecules, fragments, etc.) with enhanced sensitivity. The stable attachment of His-tagged NS2B-NS3 protease to the chip surface was achieved through His capture and amine coupling strategy. This immobilization technique provides oriented and active protein immobilization for binding assays and can be applied to different protein targets. Binding analysis was performed with the Repeated Analyte Pulses of Increasing Duration (RAPID) method, which involves the injection of samples at a single concentration with varied association times. Kinetic parameters were obtained from Creoptix Wave software (v 4.5.18).

Materials

For each section protocol method the materials are included in detail.

Protein expression and purification

2d

1 The NS2B-NS3 protease used in this assay was purified and expressed using the following protocol.

2d

Protocol

NAME

Zika Virus NS2B-NS3 protease fusion protein his10-tagged protein expression and purification: large scale 1 L cultures

CREATED BY

Michael Fairhead

Preview

Immobilization

2h

2 ZIKV-NS2B3 protease used in the concentration [M] 25 µg/µL with buffer HBS-P following the protocol bellow.

2h

Protocol

NAME

Creoptix Protein Immobilization Protocol: His-tag Capture via EDC/NHS chemistry

CREATED BY

Eda Capkin

Preview

Binding assay

2h

3 Binding assay were performed with RAPID kinetics using the following protocol. The running buffer condition is 1XHBS-P + 2% DMSO and samples were injected at [M] 10 micromolar (µM) for 5 s association and 60 s dissociation at 200 uL/min flow rate. 2h

Protocol

NAME

Kinetic assay with waveRAPID grating-coupled interferometry waveCreoptix system

CREATED BY

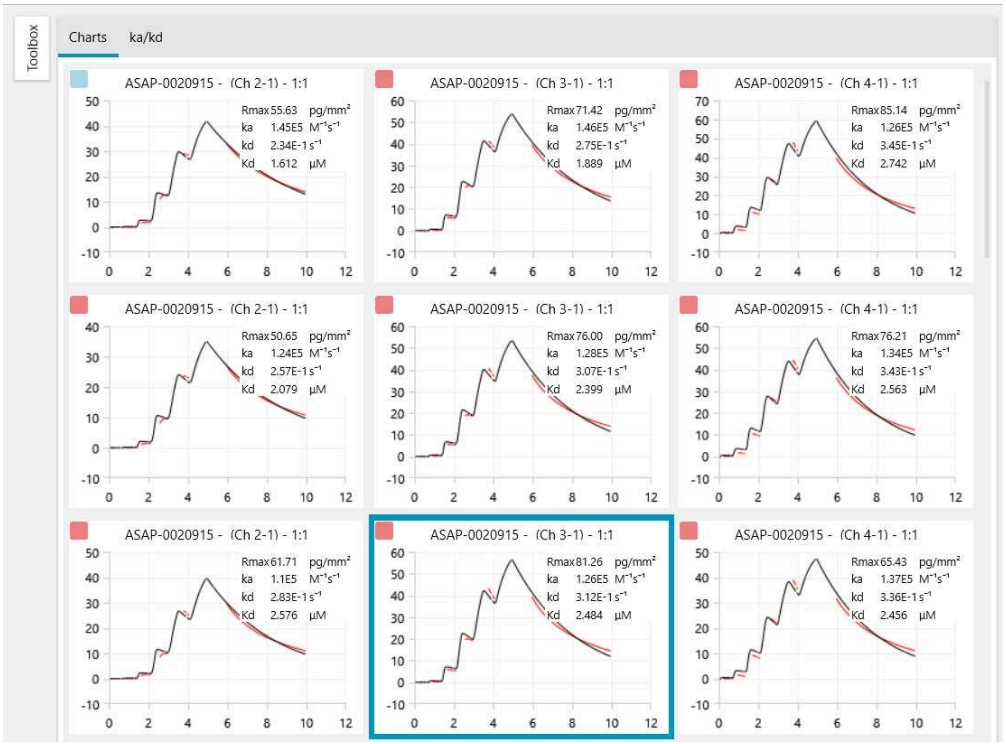
Eda Capkin

Preview

3.1 Samples are calculated: 100,000 uM*V1= 10 uM * 200 uL
20 nL is tranfered to 384 well Echo plates (max vol 100 uL). Then, 100 ul 1X HBS-P+2%DMSO added to 384 well Echo pp plate and tranferred to 100 uL containing 384 well plate(max vol 240 uL) for Creoptix.

Results expected and data analysis 2h

4 Check the variation and/or similarity of K_D values across different subtracted active flow channels (FC2-1, FC3-1, and FC4-1). 2h



Kinetic parameters (ka, kd, KD) comparison for a sample (with a low µM IC50) over subtracted active channels (FC2-1 red, FC3-1 green, and FC4-1 orange) for ZIKV-NS2B-NS3 protease

Protocol

NAME

Data Analysis waveRAPID using Creoptix WAVEsystem

CREATED BY

Eda Capkin

Preview

4.1 If there is a biochemical data (IC_{50}), compare pIC50 values vs pKD values.