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Dengue 2 NS2B-NS3 protease binding assay for compound screen using grating-coupled interferometry

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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 Dengue 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. Biotin-tagged NS2B-NS3 protease was captured on the streptavidin chip surface . 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.

Protocol materials

Dengue virus serotype 2 NS2B-NS3 protease fusion with non cleavable linker and non cleavable avi-tag **addgene Catalog #**228649

Protein expression and purification

2d

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

2d

Dengue virus serotype 2 NS2B-NS3 protease fusion with non cleavable linker and non cleavable avi-tag **addgene Catalog #**228649

Protocol

NAME

Dengue Virus Serotype 2 NS2B-NS3 protease fusion protein Avi-tagged (Biotinylated) protein expression and purification: large scale 1 L cultures

CREATED BY

Mary-Ann Xavier

Preview

Immobilization

2h

2 DENV-2 NS2B-NS3 protease used at the concentration [M] 10 µg/µL for 200 s with buffer HBS-P, following the protocol below.

2h

Protocol

NAME

Creoptix Protein Immobilization Protocol: Biotin Capture on Streptavidin Chip

CREATED BY

Eda Capkin

Preview

Binding assay

2h

3 Binding assays were performed with RAPID kinetics using the following protocol. The running buffer condition is HBS-P + 2% DMSO and samples were injected at varied concentrations depending on the response at the first trial ([M] 0.5 micromolar (µM) to [M] 25 micromolar (µM) . The samples were injected for 25 s association and 300 s dissociation at 100 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

100,000 uM*V1= 25 uM * 200 uL
50 nL

Sample volumes are transferred to 96-well plates.

The samples (at [M] 10 micromolar (µM) and [M] 25 micromolar (µM))are directly diluted with 200 µL 1X HBS-P+2%DMSO.

0.5 uM and 1 uM samples are diluted from 10 uM intermediate stocks with 1X HBS-P+2%DMSO.

Results expected and data analysis

2h

4 Check the control sample and samples binding response on both the all flow channels (FC1, FC2, FC3, and FC4) and subtracted active flow channels (FC2-1, FC3-1, and FC4-1). 2h

Protocol

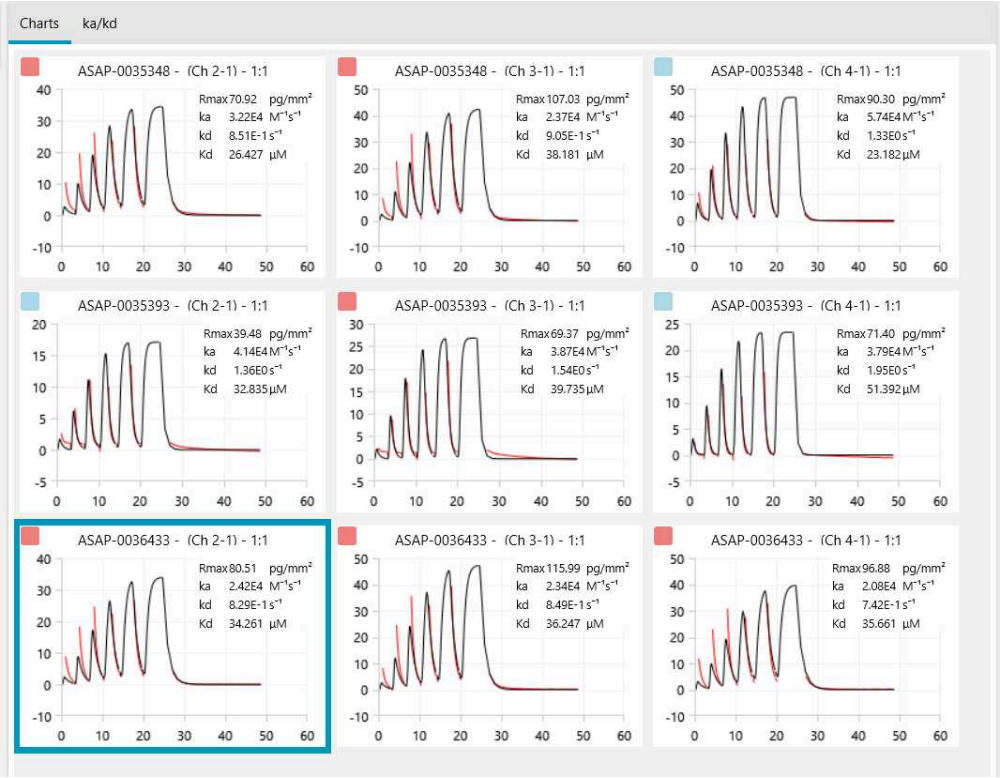
NAME

Data Analysis waveRAPID using Creoptix WAVEsystem

CREATED BY
Eda Capkin

Preview

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



Kinetic parameters (ka, kd, KD) comparison for samples over subtracted active channels (FC2-1 red, FC3-1 green, and FC4-1 orange) for DENV-2-NS2B-NS3 protease

Protocol references

1. Kartal Ö, Andres F, Lai MP, Nehme R, Cottier K. waveRAPID—A Robust Assay for High-Throughput Kinetic Screens with the Creoptix WAVEsystem. SLAS DISCOVERY: Advancing the Science of Drug Discovery. 2021;26(8):995-1003. doi:10.1177/24725552211013827.