Aug 04, 2025

Live Virus Dengue - Vero-TMPRSS2 + PGP Inhibitor - Antiviral Screening Assay

DOI
https://dx.doi.org/10.17504/protocols.io.14egn6d5ml5d/v1

Briana McGovern[1,2]

[1]Icahn School of Medicine at Mount Sinai; [2]ASAP Discovery Consortium

ASAP Discovery

Briana L McGovern
Icahn School of Medicine at Mount Sinai

DOI: https://dx.doi.org/10.17504/protocols.io.14egn6d5ml5d/v1

Protocol Citation: Briana McGovern 2025. Live Virus Dengue - Vero-TMPRSS2 + PGP Inhibitor - Antiviral Screening Assay. **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egn6d5ml5d/v1

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

Created: October 08, 2024

Last Modified: August 04, 2025

Protocol Integer ID: 109403

Keywords: antiviral, live virus, screening, drug discovery, cytotoxicity, Dengue, DENV, antiviral screening against dengue, antiviral screening assay, antiviral screening assay the following, antiviral screening, live virus dengue, pgp inhibitor, screening assay, antiviral candidate, dengue, protocol for live virus, tmprss2, pgp inhibtior, matching cytotoxicity, immunofluorescent staining, analysis of immunofluorescent staining

Funders Acknowledgements:
National Institutes of Health/National Institute Of Allergy and Infectious Diseases (NIH/NIAID)
Grant ID: U19AI171399

Disclaimer

> The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Abstract

The following is a protocol for live virus antiviral screening against Dengue in vitro. Cells are prophylactically treated with antiviral candidates that have been serially diluted to investigate activity, via IC50 values generated via analysis of immunofluorescent staining, against Dengue.
You may omit PgP inhibtior.
Each live virus screening assay is accompanied by a matching cytotoxicity screening assay to investigate drug toxicity.

Materials

10% Media
2% Media
96-well plates
96-well deep well plates
12-channel 200/300ul multichannel pipette
200/300ul filtered tips
Compounds of interest

Virus(es) of interest
PPE
reservoir

Protocol materials

Deep Well Plate (96 well) **Thermo Fisher Catalog #**10045

Formaldehyde

Safety warnings

Please be sure to wear proper Personal Protective Equipment (PPE) while performing this experiment.

Before start

Assay is performed under biosafety level 3 conditions.

Seeding

1 The day before your assay, seed 96-well plates with Vero-TMPRSS2 cells at **4,000 cells/well** using standard **10% Growth Media**.

You will need to make **one pair of plates** for every 3 compounds. One plate is the antiviral plate that will be infected, and the other is the cytoxicity plate that will measure the toxicity of the antiviral candidates.

For each pair you can test 3 compounds (in triplicate, 8 dilutions per compound), 2 DMSO controls, and 1 uninfected control in the following layout:

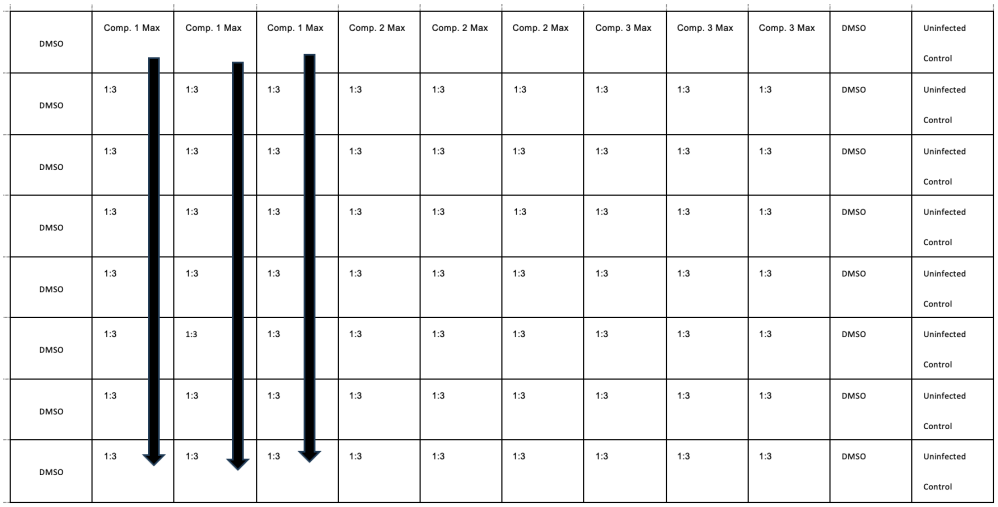

Plate Layout

Protocol

NAME

Cytotoxicity Screening Assay - Paired with Antiviral Assays

CREATED BY

Briana L McGovern

Preview

Setting up the conditions

2 Create a screening priority list ahead of time. This list will have the compounds of interest, what maximum concentration you'd like to screen at, and where the compounds are located. We recommend organizing the compounds the day before.

3 The serial dilutions will follow different protocols depending on if you will do them by hand or by using a

Equipment

Tecan D300e

Liquid Handler

Tecan

30100152

https://lifesciences.tecan.com/products/liquid_handling_and_automation/tecan_d300e_

STEP CASE

Manual 18 steps

Performing the serial dilutions manually.

If your lab does not have a Tecan D300e machine you will need to do the dilutions by hand.

4 Remove your compounds from -20 to thaw while you set up.

5 Calculate how much media you will need for the entire experiment using the following values in step 5.1

For this assay, the total volume of media that you make will need to be supplemented with 2uM PGP Inhibitor Elacridar. Elacridar should also be present in the media used for the actual infection so take that volume into account as well.

You may omit PgP inhibitor if desired.

5.1 Prepare the Deep Well Plate (96 well) **Thermo Fisher Catalog #10045** according to this diagram:

Deep Well Plate X (without tecan)

Comp. 1 Max (1100ul)	Comp. 2 Max (1110ul)	Comp. 3 Max (1110ul)	Comp. 4 Max (1110ul)	Comp. 5 Max (1110ul)	Comp. 6 Max (1110ul)	Comp. 7 Max (1110ul)	Comp. 8 Max (1110ul)	Comp. 9 Max (1110ul)	Comp. 10 Max (1110ul)	Comp. 11 Max (1110ul)	Comp. 12 Max (1110ul)
(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)
(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)
(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)
(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)
(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)
(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)
(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)	(750ul)

Deep well set up for manual serial dilutions

The first row will be the [max] of your compounds. This row will contain **1100ul** of 2% media **without DMSO**. The media for this row does not receive any DMSO since you will add drug dissolved in DMSO directly to it.

After filling the first rows, normalize the remainder of the media with DMSO.
Fill the remaining wells with **750ul 2% media supplemented with DMSO.**

These volumes are enough to treat both the antiviral and cytotoxicity plates.

6 Perform the serial dilutions:
Add the compounds of interest to the first row of the deep well (containing 1100ul media).

6.1 **mix well** and move **375ul** from row 1 to the row 2. This is your first 1:3 dilution.

Deep Well Plate X (without tecan)

Comp. 1 Max (1100ul)	Comp. 2 Max (1110ul)	Comp. 3 Max (1110ul)	Comp. 4 Max (1110ul)	Comp. 5 Max (1110ul)	Comp. 6 Max (1110ul)	Comp. 7 Max (1110ul)	Comp. 8 Max (1110ul)	Comp. 9 Max (1110ul)	Comp. 10 Max (1110ul)	Comp. 11 Max (1110ul)	Comp. 12 Max (1110ul)
(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)
(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)
(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)
(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)
(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)
(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)
(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)

Schematic of the manual serial dilutions

6.2 Repeat until the entire deep well has been done.
You now have 8 concentrations of your compounds of interest.

7 Remove the growth media from the plates. Work one pair of plates at a time to prevent drying of the cells.

Add **100ul** of the serial dilutions to the wells in the following set up: You will perform this for **both** the infection plate and the matching cytotoxicity plate

DMSO	Comp. 1 Max	Comp. 1 Max	Comp. 1 Max	Comp. 2 Max	Comp. 2 Max	Comp. 2 Max	Comp. 3 Max	Comp. 3 Max	Comp. 3 Max	DMSO	Uninfected Control
DMSO	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	DMSO	Uninfected Control
DMSO	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	DMSO	Uninfected Control
DMSO	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	DMSO	Uninfected Control
DMSO	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	DMSO	Uninfected Control
DMSO	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	DMSO	Uninfected Control
DMSO	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	DMSO	Uninfected Control
DMSO	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	DMSO	Uninfected Control

Treatment layout of the HeLa-ACE2 plates.

You should always have the plates in the correct orientation (top left corner should be A1) so that you are sure where the highest and lowest [compound] are.

8 Incubate the cells at 37 °C 5% CO_2 for 02:00:00 .

2h

Prepping for infection

1h 30m

9 Dilute Dengue stock in 2% media with 2uM elacridar for infection to an **MOI of approximately 0.15**.

MOI is chosen to facilitate about 30 percent infection in DMSO wells.

Protocol

NAME

Calculating Multiplicity of Infection (MOI)

CREATED BY

Briana L McGovern

Preview

10 Perform the mock infection on the cytotoxicity plates.

Add **50ul 2% media with elacridar** to the wells.

Protocol

NAME

Cytotoxicity Screening Assay - Paired with Antiviral Assays

CREATED BY

Briana L McGovern

Preview

Infection

1d

11 Add 50uL of diluted virus to infected wells of antiviral plate

12 Incubate at 37 °C for **6 days**

Fixing

1d

13 After **6 days,** add **100ul** [M] 10 % (v/v) Formaldehyde to all wells on the plate.

13.1 Incubate for 24:00:00 at room temperature

1d

14 In parallel, process the cytotoxicity plates.

Protocol

NAME

Cytotoxicity Screening Assay - Paired with Antiviral Assays

CREATED BY

Briana L McGovern

Preview

Staining

15 Follow the Dengue Antiviral Staining Protocol

Protocol

NAME

DENGUE Antiviral Immunofluorescence staining Protocol

CREATED BY

Briana L McGovern

Preview

Imaging

16 Image the infected plates on a plate reader. We use a Cytation1

Equipment

Cytation1	NAME
Imager	TYPE
Biotek	BRAND
n/a	SKU
https://www.agilent.com/en/product/cell-analysis/cell-imaging-microscopy/cell-imaging-multimode-readers/biotek-cytation-1-cell-imaging-multimode-reader-1623200	LINK

Analysis

17

Protocol

NAME

Generating Antiviral & Cytotoxicity Curves

CREATED BY

Briana L McGovern

Preview