Aug 04, 2025

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

DOI
https://dx.doi.org/10.17504/protocols.io.x54v92xj1l3e/v1

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Protocol Citation: Briana McGovern 2025. Live Virus Zika - Vero-TMPRSS2 + PGP Inhibitor - Antiviral Screening Assay. **protocols.io** https://dx.doi.org/10.17504/protocols.io.x54v92xj1l3e/v1

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

Created: September 25, 2024

Last Modified: August 04, 2025

Protocol Integer ID: 108370

Keywords: antiviral, live virus, screening, drug discovery, cytotoxicity, ZIKV, Zika virus, antiviral screening against zika, live virus zika, antiviral screening assay, antiviral screening assay the following, antiviral screening, pgp inhibitor, screening assay, antiviral candidate, zika, protocol for live virus, live virus, matching cytotoxicity, screening, tmprss2

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

Disclaimer

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Abstract

The following is a protocol for live virus antiviral screening against Zika 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 Zika.
You may choose to omit PgP inhibitor.
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:

Protocol

NAME

Cytotoxicity Screening Assay - Paired with Antiviral Assays

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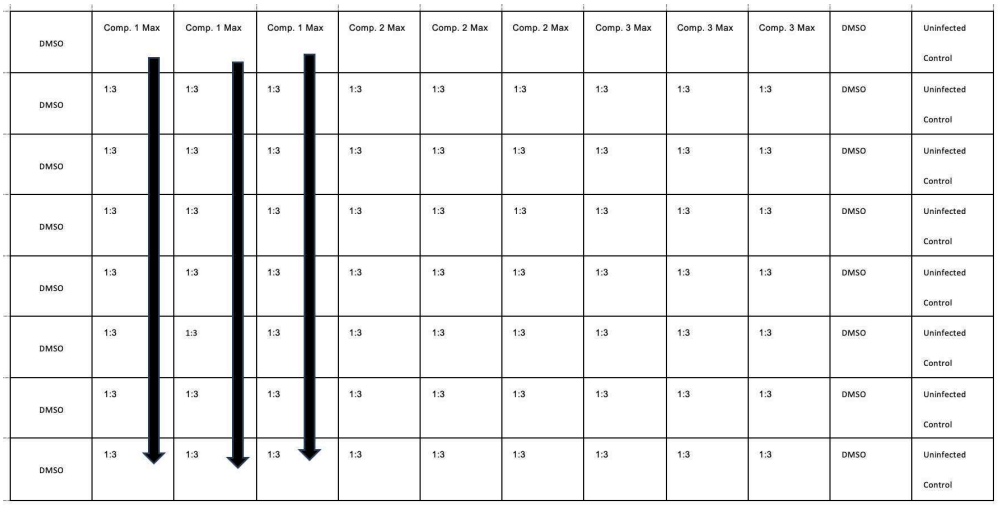
Briana L McGovern

Preview



Treatment layout

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_

Before the assay, you should prepare enough 2% Media **supplemented with 3uM elacridar (PgP inhibitor) and DMSO** for your screening. **DMSO supplementation will correspond to the highest amount of drug stock used.**

You may omit PgP inhibitor if desired.

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 choose to omit PgP inhibitor.

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.

The remaining rows of the plate will be filled 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.

Notice how the inner columns of the plate receive experimental treatment, while the outer columns are the controls.

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% CO2 for 02:00:00 .

2h

This pretreatment time frame could differ depending on what sort of compounds you'd like to test.

Prepping for infection

1h 30m

9 Dilute ZIKV stock in 2% media with 2uM elacridar (or omit if desired) for infection to an **MOI of approximately 0.05**.

MOI is chosen to facilitate about 30% infection in the DMSO control wells.

Protocol

NAME

Calculating Multiplicity of Infection (MOI)

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Briana L McGovern

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10 Perform the mock infection on the cytotoxicity plates.

Add **50ul 2% media with elacridar** (or omit) to the wells.

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Cytotoxicity Screening Assay - Paired with Antiviral Assays

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Infection

1d

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

12 Incubate at 37 °C for 24:00:00 1d

Fixing

1d

13 After 24 hours, add **100ul** [M] 10 % (v/v) Formaldehyde to all wells on the plate.

13.1 Incubate for 24:00:00 at room temperature inside the bags 1d

14 In parallel, process the cytotoxicity plates.

Protocol

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Cytotoxicity Screening Assay - Paired with Antiviral Assays

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Staining

15 Follow the ZIKA Antiviral Staining Protocol

Protocol

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ZIKA Antiviral Immunofluorescence staining Protocol

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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

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Generating Antiviral & Cytotoxicity Curves

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