Mar 19, 2025

Cytotoxicity Screening Assay - Paired with Antiviral Assays

DOI
https://dx.doi.org/10.17504/protocols.io.bp2l62mozgqe/v1

Briana McGovern[1,2], Kris White[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.bp2l62mozgqe/v1

Protocol Citation: Briana McGovern, Kris White 2025. Cytotoxicity Screening Assay - Paired with Antiviral Assays. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp2l62mozgqe/v1

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

Created: August 07, 2024

Last Modified: March 19, 2025

Protocol Integer ID: 104824

Keywords: cytotoxicity, antiviral, antiviral assay, antiviral assays the following, antiviral screening, cytotoxicity screening assay, mts assays in uninfected cell, live virus antiviral screening, antiviral screenings for the family, antiviral plate, pgp inhibitor in the assay, antiviral candidate, cytotoxicity protocol, cytotoxicity of the compound, cytotoxicity plate, cytotoxicity, mts assay, pgp inhibitor, uninfected cell, assay, live virus, infection, virus, infection media

Funders Acknowledgements:
National Institutes of Health/National Institute Of Allergy and Infectious Diseases (NIH/NIAID)
Grant ID: 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 cytotoxicity protocol to be paired with a live virus antiviral screening in vitro. Cells are prophylactically treated with antiviral candidates that have been serially diluted to investigate activity, via CC50 values generated via analysis of MTT/MTS assays in uninfected cells.
You may opt to include PgP inhibitor in the assay by adding 2uM into the infection media.
For antiviral screenings for the family of virus you are working on, you will set up both an antiviral plate (infected) and a cytotoxicity plate (uninfected). The cytotoxicity plate will measure the cytotoxicity of the compounds on the cells.

Protocol materials

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

Safety warnings

Always wear appropriate PPE for this protocol
Refer to Material Safety Data Sheets for additional safety and handling information.

Seeding

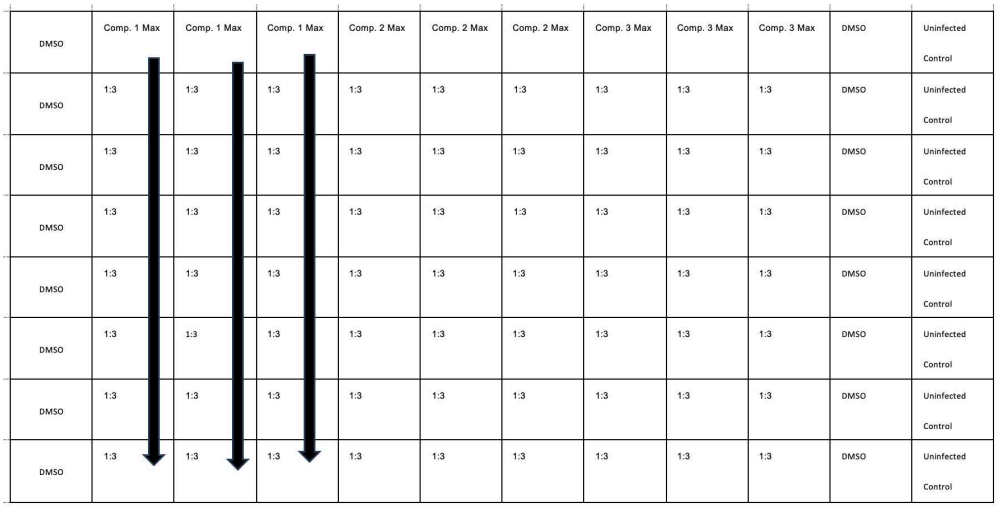
1 This assay is typically run in parallel to the **antiviral screening protocol**, so you will end up having 1 pair of plates for every 3 compounds that you want to test. **The pair will be identical**; **one will be infected** to test antiviral activity, while **the other will remain uninfected** to test cytotoxicity.

The cells and their density will change depending on which virus you are screening against.
Coronaviruses (SARS-CoV-2 & MERS-CoV): Vero-TMPRSS2
Influenzas: A549
Picornaviruses: Rd (Rhabdomyosarcoma)
Flaviviruses: Vero-E6/TMPRSS2

Refer to **this SARS-CoV-2 antiviral screening assay protocol** for more information.

2 The day before your assay you will seed your cells of interest. You will need 2 plates for every 3 compounds.

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:



Treatment layout of the plates.

Setting up the conditions

3 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.

4 The serial dilutions will follow different protocols depending on if you will do them by hand or by using a TecanD300e.

Before the assay, you should prepare enough 2% Media **supplemented with DMSO** for your screening. **DMSO supplementation will correspond to the highest amount of drug stock used.**

Each deep hold 12 compounds, and will need 80 mL.

STEP CASE

Manual From 14 to 15 steps

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

6 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.

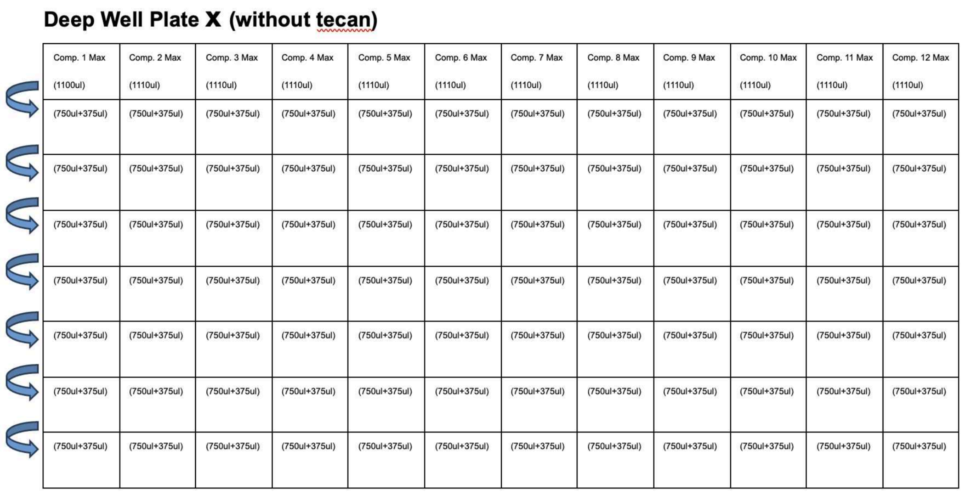
The remaining rows of the plate will be filled with **750ul 2% media + supplemented with DMSO**

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

7 Perform the serial dilutions:

Add the compounds of interest to the first row of the deep well (containing 1100ul media).

7.1 **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)

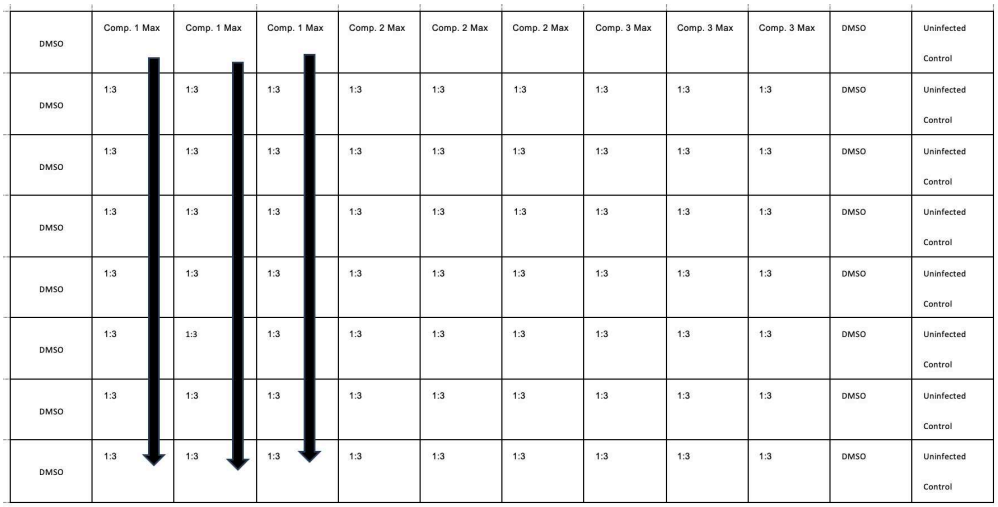
Comp. 1 Max (1100ul)	Comp. 2 Max (1110ul)	Comp. 3 Max (1110ul)	Comp. 4 Max (1110ul)	Comp. 5 Max (1110ul)	Comp. 6 Max (1110ul)	Comp. 7 Max (1110ul)	Comp. 8 Max (1110ul)	Comp. 9 Max (1110ul)	Comp. 10 Max (1110ul)	Comp. 11 Max (1110ul)	Comp. 12 Max (1110ul)
(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)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

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

8 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



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.

9 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, but 2 hours is standard.

Mock Infection

10 While these cytotoxicity plates will not be infected with virus, they need to be mock infected to match the antiviral plates.

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

11 After the infection timeline (which will vary depending on which virus you are screening against), you will fix both the antiviral and cytotoxicity plates. The method you use for the cytotoxicity plates will depend on which reagents you have.

STEP CASE

Roche MTT Kit 6 steps

Sigma #11465007001

12 add 10ul MTT reagent.

12.1 Incubate 3-4 hours at 37C

13 Add 100ul SDS reagent and incubate at RT ON

Reading the plates

14 Read the plates using a plate reader. We use the Cytation1.
read at 560nm
read again at 750nm

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

14.1 Export the data to excel

Analysis

15 use https://www.protocols.io/edit/generating-antiviral-amp-cytotoxicity-curves-diqi4due