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iPSC Pneumocyte Live Virus SARS-CoV-2 Cytotoxicity Screening Assay

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We use this protocol and it's working

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Disclaimer

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Abstract

These immunofluorescence-based antiviral assays rely on identification of infected cells through immunostaining the NP (influenza virus), N (SARS-CoV-2) proteins, or any relevant viral antigen at 488nM and counterstaining with DAPI. Infected cells (488nM) and total cells (DAPI) are identified and quantified by a plate cytometer (Biotek Cytation 1). Compounds are tested in a triplicate 8-point dose-response with concurrent cytotoxicity assays (MTT Roche/MTS Promega) in uninfected cells. These assays can be run on any adherent cell type, such as standard cell lines and iPSC-derived cell types. DMSO infection controls (negative) and uninfected cell controls (positive) should be included on every plate. The DMSO controls will establish 100% infection and the uninfected wells 0% infection for each plate. Relevant control compounds should be included as controls for every run. The effect p-gp inhibitors on known p-gp subtrates should be explored in all new cell types used.

These assays accurately reflect the antiviral activity of compounds in medium-to-high throughput, depending on the level of automation.

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.

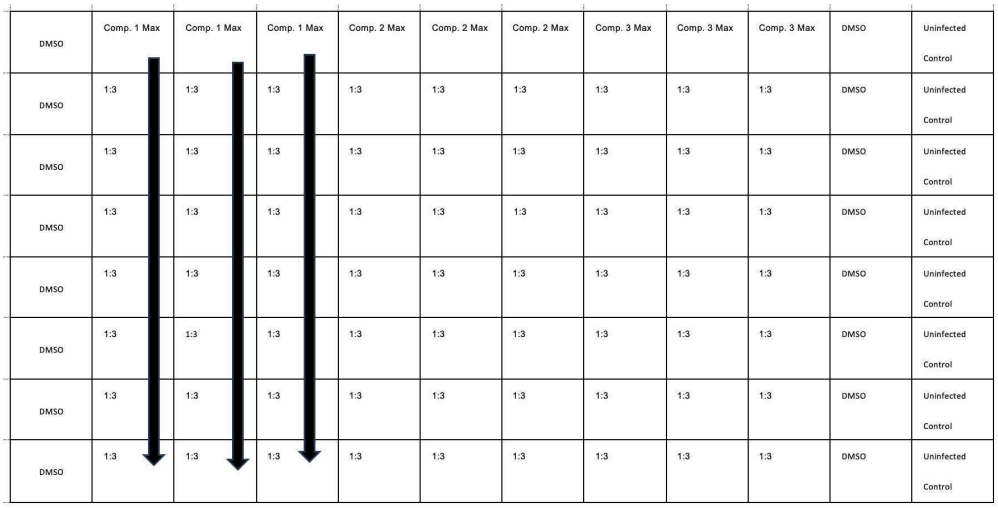
Plates

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.

2 Obtain pneumocyte cells seeded in 96-well plates and appropriate media.

Total amount of plates should be divided in half - one for antiviral screening and one for **antiviral screening**.

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



Treatment layout

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 **supplement the provided pneumocyte media with DMSO** for your screening. DMSO supplementation will correspond to the highest amount of

drug stock used.

STEP CASE

Manual

From 13 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, **by hand,** from the plates. The pneumocytes are very sensitive so it's best to aspirate the media by hand rather than vacuum line. Work one pair 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

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 24 hours post infection, 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