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Live Virus SARS-CoV-2 - iPSC Pneumocyte - Antiviral Screening

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ASAP Discovery

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We use this protocol and it's working

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Disclaimer

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Abstract

The following is a protocol for live virus antiviral screening against SARS-CoV-2 in iPSC pneumocyte cells. iPSC pneumocytes are prophylactically treated with antiviral candidates that have been serially diluted to investigate activity, via IC50 values generated via analysis of immunofluorescent staining, against SARS-CoV-2. Each live virus screening assay is accompanied by a matching cytotoxicity screening assay to investigate drug toxicity.

Guidelines

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

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

Assay is performed under biosafety level 3 conditions.

Plates

1 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 cytotoxicity screening.

Protocol

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

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Preview

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:

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

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 in storage. We recommend organizing the compounds the day before.

These conditions will be different depending on if you are going to perform the serial dilutions manually, or using the Tecan D300e (which is essentially a drug printer).

The protocol will fork into these two methods below.

3 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 21 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 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, based on your testing plan, to the first row of the deep well.

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)

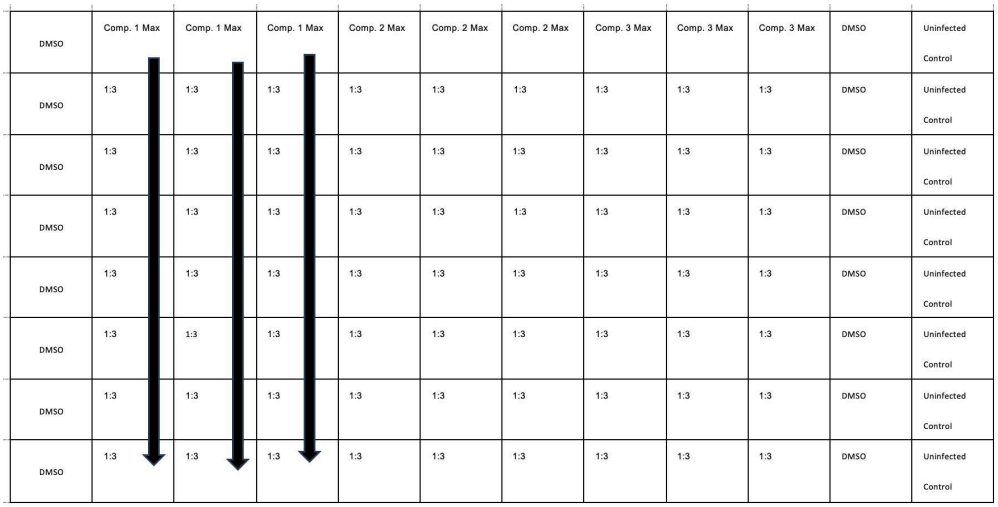
Comp. 1 Max (1100ul)	Comp. 2 Max (1110ul)	Comp. 3 Max (1110ul)	Comp. 4 Max (1110ul)	Comp. 5 Max (1110ul)	Comp. 6 Max (1110ul)	Comp. 7 Max (1110ul)	Comp. 8 Max (1110ul)	Comp. 9 Max (1110ul)	Comp. 10 Max (1110ul)	Comp. 11 Max (1110ul)	Comp. 12 Max (1110ul)
(750ul+375ul)	(750ul+375ul)	(750ul+375ul)	(750ul+375ul)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



Treatment layout

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 concentrations of 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 After approximately 01:30:00 you should begin prepping to head up to the BSL-3 facility, where you will infect the plates with SARS-CoV-2.

1h 30m

9.1 Calculate the dilution of the virus(es) of interest and bring the calculations with you.

Protocol

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Calculating Multiplicity of Infection (MOI)

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9.2 Prepare your materials for entering the facility. Tips, media, PPE etc.

10 Before heading up to infect the plates, you should perform the **mock infection on the cytoxicity plates**. We want both plates to match so that we can compare them later.

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

Protocol

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

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Infection

11 Go to the BSL-3 facility. Perform the entering procedures.

11.1 Retrieve your virus(es) from the -80.
Thaw your virus(es) and create your dilution(s)

11.2 Using the reservoir, infect your plates (except the uninfected control column) with 50ul of the virus dilution.

12 Incubate at 37 °C for 24:00:00

1d

Fixing

1d

13 After 24 hours, add **100ul** [M] 1 % (v/v) Formaldehyde to all wells on the plate.

Spray each lid gently with ethanol and replace.

Double bag the plates in red biohazard bags to bring them out of the facility.

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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Staining

15 Follow the SARS-CoV-2 staining protocol

Protocol

NAME

SARS-CoV-2 Antiviral Immunofluorescence staining Protocol

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Imaging

16 Image the infected plates on the

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

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Protocol

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Generating Antiviral & Cytotoxicity Curves

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