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Live Virus SARS-CoV-2 - HeLa-ACE2 - Antiviral Screening Assay

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

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Disclaimer

Assay is performed under biosafety level 3 conditions.

Abstract

The following is a protocol for live virus antiviral screening against SARS-CoV-2 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 SARS-CoV-2.
You may opt to include PgP inhibitor in the assay by adding 2uM into the infection media.
Each live virus screening assay is accompanied by a matching cytotoxicity screening assay in uninfected cells 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

Formaldehyde

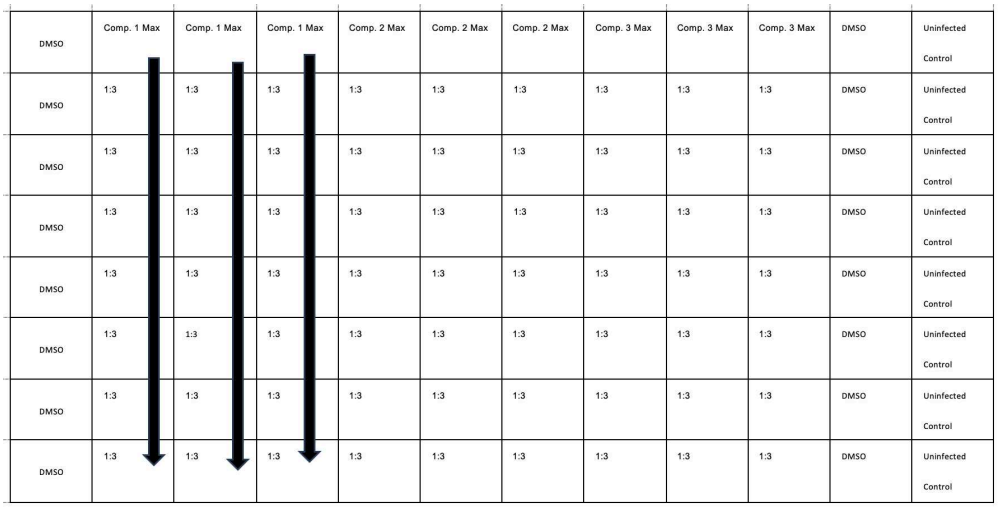
Deep Well Plate (96 well) **Thermo Fisher Catalog #**10045

Seeding

1 The day before your assay, seed 96-well plates with **HeLa-ACE2** 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:



Treatment layout

Protocol

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Cytotoxicity Screening Assay - Paired with Antiviral Assays

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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 DMSO** for your screening. **DMSO supplementation will correspond to the highest amount of drug stock used.**

Include 2uM PgP inhibitor in this infection media if you'd like.

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 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% CO_2 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.

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.

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Cytotoxicity Screening Assay - Paired with Antiviral Assays

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Infection

1d

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.

11.3 Incubate at 37 °C for 24:00:00

1d

Fixing

1d

12 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.

12.1 Incubate for 24:00:00 at room temperature inside the bags

1d

13 In parallel, process the cytotoxicity plates.

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Staining

14 Follow the SARS-CoV-2 Antiviral Staining Protocol

Protocol

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SARS-CoV-2 Antiviral Immunofluorescence staining Protocol

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Imaging

15 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

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Generating Antiviral & Cytotoxicity Curves

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