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Live Virus MERS-CoV - Vero-TMPRSS2 + Pgp Inhibitor- Antiviral Screening Assay

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We use this protocol and it's working

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Abstract

The following is a protocol for live virus antiviral screening against MERS-CoV EMC2012 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 MERS-CoV.

You may opt to remove PgP inhibitor in the assay.

You may opt to use VeroE6 cells rather than Vero-TMPRSS2 cells.

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

 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

Always wear appropriate PPE for this protocol

Refer to Material Safety Data Sheets for additional safety and handling information.

Seeding

- 1 The day before your assay, seed 96-well plates with Vero-E6-TMPRSS2 (or you may opt for plain VeroE6) cells at **2,000 cells/well**



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 cytotoxicity plate that will measure the toxicity of the antiviral candidates.

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 of the plates.

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 3uM elacridar (PgP inhibitor) and DMSO** for your screening. **DMSO supplementation will correspond to the highest amount of drug stock used.**

you may omit elacridar from your run if you wish.

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	Comp. 2 Max	Comp. 3 Max	Comp. 4 Max	Comp. 5 Max	Comp. 6 Max	Comp. 7 Max	Comp. 8 Max	Comp. 9 Max	Comp. 10 Max	Comp. 11 Max	Comp. 12 Max
(1100ul)	(1110ul)	(1110ul)	(1110ul)	(1110ul)	(1110ul)	(1110ul)	(1110ul)	(1110ul)	(1110ul)	(1110ul)	(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	Comp. 2 Max	Comp. 3 Max	Comp. 4 Max	Comp. 5 Max	Comp. 6 Max	Comp. 7 Max	Comp. 8 Max	Comp. 9 Max	Comp. 10 Max	Comp. 11 Max	Comp. 12 Max
(1100ul)	(1110ul)	(1110ul)	(1110ul)	(1110ul)	(1110ul)	(1110ul)	(1110ul)	(1110ul)	(1110ul)	(1110ul)	(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 After approximately 01:30:00 you should begin prepping to head up to the eBSL-3 facility, where you will infect the plates with MERS-CoV.

1h 30m

- 9.1 Calculate the dilution of the virus of interest and bring the calculations with you.





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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 cytotoxicity 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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Infection

1d

11 Go to the eBSL-3 facility and perform the necessary procedure to enter. Plan ahead to give yourself extra time to enter the facility, you will need 30 minutes minimum.

11.1 Perform the necessary set up for working in the enhanced facility.

Retrieve your virus(es) from the -80.





Thaw your virus and create your dilution

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** 1 % (v/v) Formaldehyde to all wells on the plate.



Double bag the plates in red biohazard bags, spray the outside of the bags with quatricide, place into the carrier, spray the carrier with quatricide, and place the carrier into the exit room.

12.1 **You are not permitted to access the plates for a longer period of time** than the SARS-CoV-2 fix plates.

2d

The carrier will remain in this exit room to incubate for minimum 48:00:00 at room temperature inside the bags.



13 In parallel, fix and image the cytotoxicity plates (at BSL-2) according to the cytotoxicity protocol

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Staining

14 Follow the MERS-CoV Antiviral Staining Protocol

Protocol

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MERS-CoV 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

16



Protocol

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Generating Antiviral & Cytotoxicity Curves

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