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Rapid Cell-Based SARS-CoV-2 Neutralization Assay with Ha-CoV-2 Pseudovirus

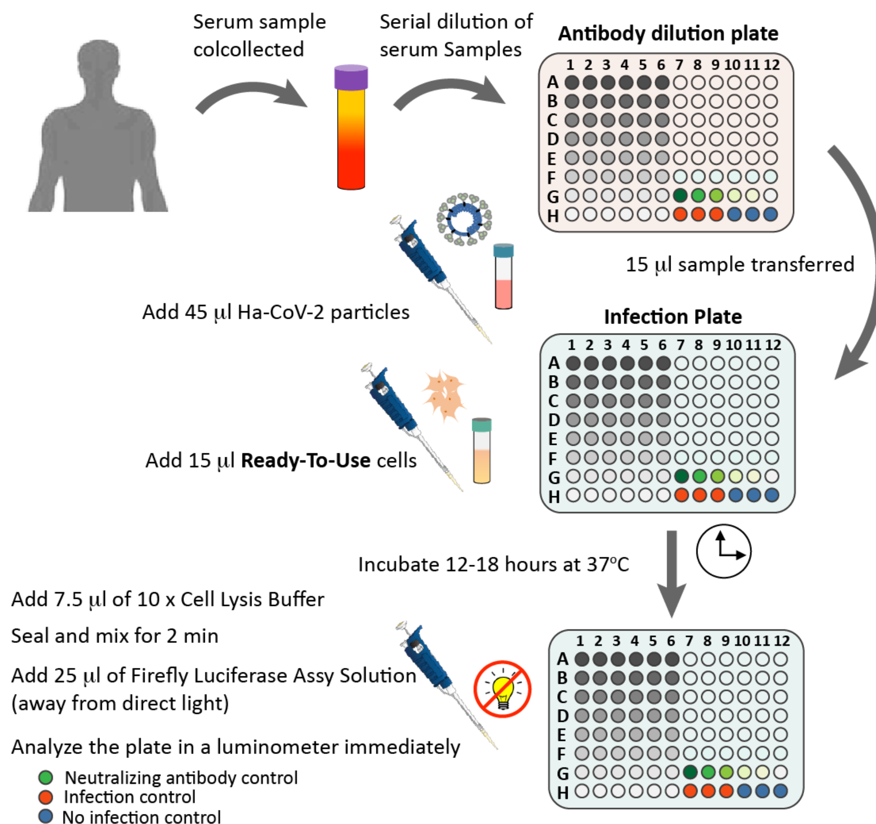
Utilizing our proprietary rapid-expressing hybrid alphavirus
Optimized for accuracy and simple-to-use procedures

Our proprietary technology is fundamentally superior to others due to the implementation of a newly developed Ha-CoV-2 particle. Ha-CoV-2 is a hybrid SARS-CoV-2 virus-like particle (VLP) that encapsulates an alphavirus-derived RNA genome for rapid expression of reporter genes (luciferase or GFP) in target cells (*Hetrick et al., 2022*). Different from commonly used S protein pseudotyped lenti- or vesicular stomatitis virus (VSV)-pseudoviruses, Ha-CoV-2 is assembled with all 4 structural proteins (S, M, N, and E) of SARS-CoV-2, and a reporter genome derived from an alphavirus-based vector for rapid and robust (high signal-to-noise ratio) expression of reporter genes. Ha-CoV-2 represents a major technology advancement in the development of SARS-CoV-2 pseudoviruses, and serves as platforms for rapid and robust quantification of neutralizing antibodies, viral variants, and antiviral drugs (*Dabbagh et al., 2021; He et al., 2021*).

Assay Highlights (Higher accuracy and simpler than ELISA)

- Ready-To-Use pre-counted target cells shipped to customers, just thaw and go, no needs for cell culture.
- No single washing steps - simply mix antibody, virus, and cells, and then read results.
- High signal-to-noise ratio (10^2 - 10^3).
- High reproducibility and consistency between wells and sample replicates.
- Proprietary Virongy Ha-CoV-2 Infection Medium eliminates the need for CO_2 cell culture incubator.

Quick User Guide (No Wash, Ready-to-Use cells)



I. Equipment, Supplies and Reagents

Equipment Not Included	Recommended (Vendor, Cat No)
Sterile Reagent Reservoir	Fisher, Cat# 03-449-255
AB Mixing Plate: Olympus 96-Well PCR Plate, Non-Skirted UltraThin Wall, Natural, 25 Plates/Unit	Genesee Scientific, Cat# 24-300
Infection Plate: 96-Well Tissue Culture Plate, Greiner Bio-One (With Lid, µClear® White Flat Round, Chimney)	VWR, Cat# 82050-758
Luciferase Reader: GloMax Discover Microplate Reader	Promega, Cat# GM3000
Reagents Included	
Rapid Cell-Based SARS-CoV-2 Neutralizing Antibody AssayKit 1. HEK293T(ACE2/TMPRSS2)-Ready-To-Use target cells 2. Ha-CoV-2(Luc) VLP 3. SARS-CoV-2 Standard Neutralizing Antibody Control 4. 10 x Lysis Buffer 5. D-Luciferin Solution 6. Firefly Luciferase Buffer Solution 7. Virongy Infection Media	Virongy, Cat# RCSNAK-02

II. Procedure

A. Serum dilution and incubation with Ha-CoV-2 pseudovirus

1. Reserve 6 wells for controls on the plate: **Infection Control** (virus + cell only) and **No Infection Control** (cells only).
2. In the antibody dilution plate, prepare antibody samples and **Virongy Infection Media** according to the chart below (example for one antibody sample) and repeat for all antibody samples and the Virongy **SARS-CoV-2 Standard Neutralizing Antibody Control**. Place **40 µL Virongy Infection Media** in rows **A-F** of the dilution plate.
3. Perform a serial dilution with the antibody samples according to the chart. Add in **10 µL** of the antibody sample to well **A**. Transfer **10 µL** from row **A** to row **B** and mix well using a higher volume setting on the pipette, pipette and mix the antibody and medium thoroughly before transferring to the next well. Continue to dilute until the 6th dilution is complete. (**Note:** Be sure to change pipette tips between well transfers and ensure that antibody and medium are mixed thoroughly.)

Well	Antibody dilution Plate
A1 (antibody, 1:5 dilution)	10 µL of sample + 40 µL Virongy Infection Media
B1 (antibody, 1: 25 dilution)	10 µL of well A1 + 40 µL Virongy Infection Media
C1 (antibody, 1: 125 dilution)	10 µL of well B1 + 40 µL Virongy Infection Media
D1 (antibody, 1:625 dilution)	10 µL of well C1 + 40 µL Virongy Infection Media
E1 (antibody, 1: 3,125 dilution)	10 µL of well D1 + 40 µL Virongy Infection Media
F1 (antibody, 1:15,625 dilution)	10 µL of well E1 + 40 µL Virongy Infection Media

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4. In the infection plate, add **45 µL** of **Ha-CoV-2 pseudovirus** into sample wells and the **Infection Control** wells (cell + virus only). Reserve 3 wells (no virus added) for the **No Infection Control** wells (cells only).
5. In the **Infection Control** wells, add **15 µL** of **Virongy Infection Media**.
6. In the **No Infection** Control wells, add **60 µL** of Virongy Infection Media.
7. Using a multichannel pipette, add **15 µL** of each antibody dilution into the respective virus containing sample wells of the Infection plate and mix well. (**Note:** Each antibody sample should be tested in duplicate on the same infection plate to ensure accurate results.)
8. Cover the plate and incubate the antibody and Ha-CoV-2 pseudovirus for **15-30 minutes** at room temperature while preparing the cells.

B. Target cell preparation

***Media must be warmed at 37°C before adding to the cells*

Virongy's **HEK293T(ACE2/TMPRSS2) Ready-To-Use cells** from dry ice box or from -80°C freezer:

1. Quickly thaw the **Ready-To-Use target cells** in a 37°C water bath.
2. Once thawed, immediately transfer the tubes contents to a tube containing **5 mL** of pre-warmed **Virongy Infection Medium**, mix gently.
3. Centrifuge the cells for **5 minutes** at **1,200 rpm** and discard the supernatant. Resuspend the cells in **2 mL** of pre-warmed **Virongy Infection Medium**.

C. Infection Procedure

1. Aliquot 15 µL of the resuspended **Ready-To-Use target cells** into each well of the infection plate. Each well should receive around **2.5 x 10^4 cells** in **15 µL**. (**Note:** If using a multichannel pipette, pour the resuspended target cells into a sterile reservoir, and pipette, mix, and then transfer **15 µL** of the cells into each well of the infection plate)
2. Cover and note the time of infection. Place the plate at **37°C** for **16-18 hours** (Overnight). (**Note:** this incubation does not have to be in a CO_2 incubator)

D. Luciferase assay protocol (Post-Infection)

Before starting: Thaw the **10 x Cell Lysis Buffer**, Firefly **Luciferase Buffer Solution**, and the **D-Luciferin Solution** in the biosafety cabinet before preparing the reagents mixtures. Keep all reagents on ice once thawed. Turn off the lights in the vicinity of the luminometer before loading the plate.

1. Following the incubation of the Infection Plate, add **7.5 µL** of the **10 x Cell Lysis Buffer** directly to each well and mix thoroughly by orbital shaking for **2 mins.** Allow cells to lyse for at least **5 minutes** at room temperature.
2. In a dark location, away from direct light, prepare the Firefly **Luciferase Assay Solution** by mixing the **D-Luciferin Solution** with the Firefly **Luciferase Buffer Solution** in a **1:50** ratio. For a full 96 well plate prepare **3 mL** of the luciferase substrate solution by mixing **60 µL** of the **D-Luciferin Solution** with **2940 µL** of the Firefly **Luciferase Buffer Solution**. (**Note:** Use the Firefly **Luciferase Assay Solution** within **30 minutes** of preparation. Do not reuse the Firefly **Luciferase Assay Solution**.)
3. Add **25 µL** of the Firefly **Luciferase Assay Solution** to the cell lysates. Mix the plate by orbital shaking for **1 min.** (**Note:** For a more accurate luciferase reading, we recommend using a programable injector when possible to add the Firefly **Luciferase Assay Solution**, so the timing of the assay can be precisely controlled.)

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4. The plate should be analyzed by a luminometer within **10-15 minutes** of adding the Firefly **Luciferase Assay Solution**. (**Tip:** Run the plate reader in a dark room to reduce any background signal; more Firefly **Luciferase Assay Solution** can add to generate a longer and stronger luciferase signal. Calculate the results by subtracting the background signal determined from the average of the "Control No Infection" wells.

III. Analyzing the Data

A. Calculate the Percent Infection

1. Calculate the average luciferase signal of the **No Infection Control** wells which is the background signal.
2. Calculate the average luciferase signal of the Infection Control wells and subtract the background signal. This represents **100%** percent infection.
3. Calculate the average luciferase signal of each Sample well, and then subtract the background signal. Calculate the percentage of infection for each sample well by dividing the sample average by the Infection Control average and multiplying by **100**. (**Note:** If any values fall below zero change to **0%** and if any are above one hundred change to **100%**)

B. Calculate the Log concentration and the IC_{50} using available software

1. The IC_{50} calculation can be performed with any software of choice. We recommend using Prism from GraphPad.

References

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