Viral Titration of SARS-COV-2 by Plaque Assay (Semi-Solid Agarose)

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ABSTRACT

This protocol outlines the process of plaque assay for the viral titration of SARS-CoV-2.

ATTACHMENTS

SARSCoV2_SemiSolid_PlaqueAssay.docx

MATERIALS

- Ethyl Alcohol Sigma Catalog #E7023
- MEM Thermo Fisher Catalog #11095080
- UltraPure®&trade; Agarose Thermo Fisher Catalog #16500500
- DMEM, high glucose, GlutaMAX™ Supplement, pyruvate Thermo Fisher Catalog #31966047
- Gibco™ Trypsin-EDTA (0.05%) phenol red Fisher Scientific Catalog #11580626
- Gibco™ Fetal Bovine Serum qualified One Shot™ format Fisher Scientific Catalog #A3160802
- Corning™ Costar™ Flat Bottom Cell Culture Plates (24 well) Fisher Scientific Catalog #10732552
- Corning™ Costar™ Clear Polystyrene 96-Well Microplates 330µL With Lid Fisher Scientific Catalog #10360691
- Formalin solution neutral buffered 10% Sigma Aldrich Catalog #HT501128-4L
- Crystal violet solution Sigma Aldrich Catalog #HT90132
SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

BEFORE START INSTRUCTIONS

1. Wear gloves during the entirety of the procedure.
2. Use filtered tips only.
3. Change tips as many times as possible.
4. Decontaminate all tips and pipettes using a solution of diluted bleach.

Preparing Plaque Overlay (2x)

1. Mix 455 mL MEM with 20 mL FBS.
2. Prewarm the media to 37 °C.

3. Dissolve 0.6 g Agarose in 30 mL H2O.

Note: The final concentration will be 2% in the 2x overlay.

4. Melt Agarose in the microwave until liquified.

5. Quickly add 25 mL melted Agarose to the prewarmed media from Step 2.

5.1. Close the bottle containing the Agarose/media mixture and shake vigorously for 00:00:30, then again for 3-4 more time over the 00:10:00 time period.

6. Let the solution cool at Room temperature.

Note: Solution can be stored at 4 °C and can be reused when needed.
Day 1

7 Plate 24 well plates with $7.5 \times 10^4$ Vero E6 per well in 10% FBS/DMEM.

8 Let cells grow Overnight at $37 \, ^\circ C$.

Day 2 - Viral Dilutions

9 Set up a 96-well plate in order to dilute your viral solution (serial dilutions).

10 In each well, put $270 \, \mu L$ MEM (serum-free).

11 Add $30 \, \mu L$ viral solution in the first row (A) and mix thoroughly.

Note

Steps 11 to 22 are to be performed in a BSL-3 laboratory/setting.

12 Discard your tips.

13 Transfer $30 \, \mu L$ previous mix (solution in row A) to the second row (B).
Repeat that step from row C until row H (SARS-CoV-2 often reaches titers to $10^6$).

**Day 2 - Viral Infection**

15 Transfer **250 µL** each dilution into each well.

16 Incubate at **37 °C** for **01:00:00**. Every **00:15:00**, rock the plate a bit.

17 While cells are incubating with virus, prewarm 2x overlay media.
After 1 hour absorption time, add 250 µL 2x overlay media on top of the 250uL of inoculum (final volume 500uL per well).

Incubate at 37 °C for ~ 65:00:00.

Prepare a solution of crystal violet diluted in EtOH as follows: 100 mL crystal violet + 200 mL EtOH + 700 mL water.

Put about 00500 µL formalin 4% into each well on top of the overlay media.

Let the well-plate sit for 00:20:00 to 00:30:00 at Room temperature.

Remove media from the well plate.

Add a solution of 0.25% Crystal Violet and 20% Ethanol in water to each well in order to stain viable cells.

Let the well-plate sit for 00:05:00 at Room temperature.
26  Plunge the well-plate into a first bucket filled with 1% bleach diluted in water.

27  In order to properly clean the plate, plunge the well-plate into a second bucket filled with water.

28  Count plaques against a white background.