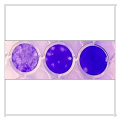

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Viral Titration of SARS-COV-2 by Plaque Assay (Semi-Solid Agarose)

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1 Works for me dx.doi.org/10.17504/protocols.io.be4zjgx6

Coronavirus Method Development Community

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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

NAME	CATALOG #	VENDOR
Ethyl Alcohol	E7023	Sigma
MEM	11095080	Thermo Fisher
UltraPure™ Agarose	16500500	Thermo Fisher
DMEM, high glucose, GlutaMAX™ Supplement, pyruvate	31966047	Thermo Fisher
Gibco™ Trypsin-EDTA (0.05%) phenol red	11580626	Fisher Scientific
Gibco™ Fetal Bovine Serum qualified One Shot™ format	A3160802	Fisher Scientific
Corning™ Costar™ Flat Bottom Cell Culture Plates (24 well)	10732552	Fisher Scientific
Corning™ Costar™ Clear Polystyrene 96-Well Microplates 330µL With Lid	10360691	Fisher Scientific
Formalin solution neutral buffered 10%	HT501128-4L	Sigma Aldrich
Crystal violet solution	HT90132	Sigma Aldrich
VERO C1008 [Vero 76 clone E6 Vero E6] (ATCC® CRL-1586™)	CRL-1586	ATCC

MATERIALS TEXT

Item	Reference	Storage conditions
Agarose (Thermofisher)	16500500	RT
DMEM (Thermofisher)	31966047	4°C
Trypsin (Gibco)	11580626	4°C
MEM (Thermofisher)	11095080	4°C
Fetal Bovine Serum (Thermofisher)	A3160802	-20°C
24 well plates (Thermofisher)	10732552	RT

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96 well plates round bottom (Thermofisher)	10360691	RT
ETHANOL ABSOLUTE EXTRA PURE	24103-5L-R	RT
FORMALIN SOLUTION, NEUTRAL BUFFERED, 10%	HT501128-4L	RT
CRYSTAL VIOLET SOLUTION	HT90132-1L	RT

SAFETY WARNINGS

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

BEFORE STARTING

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** .

The final concentration will be 4% in the 2x overlay.

2 Prewarm the **media** to **37 °C** .

3 Dissolve **0.6 g Agarose** in **30 ml H2O** .

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**.

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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** .

Solution can be stored at **4 °C** and can be reused when needed.

Day 1

7 Plate 24 well plates with 7.5x10^4 **Vero E6** per well in 10% FBS/DMEM.

8 

Let cells grow **Overnight** at **37 °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 µl MEM (serum-free)** .

11 

Add **30 µl viral solution** in the first row (A) and mix thoroughly.

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

12 Discard your tips.

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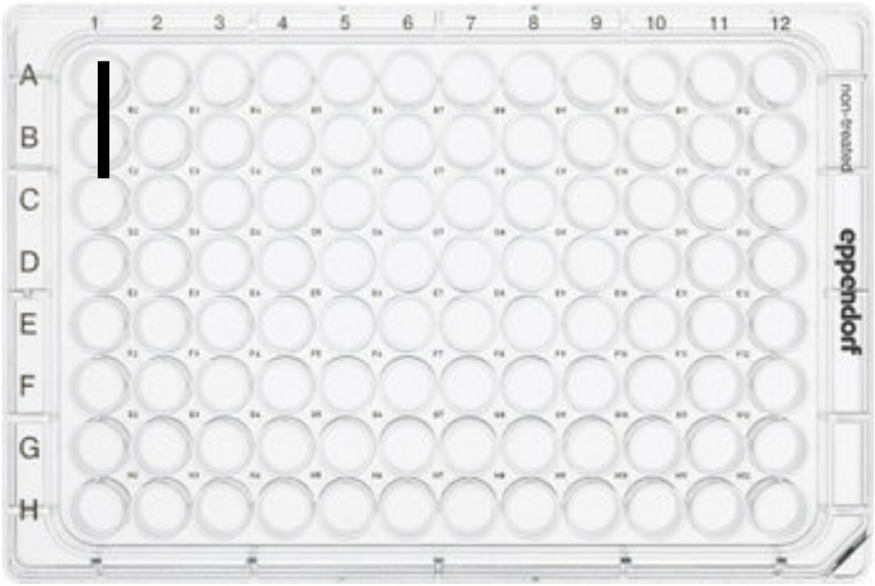
13

Transfer **30 µl previous mix (solution in row A)** to the second row (B).



14

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.

18

After 1 hour absorption time, add **250 µl 2x overlay media** on top of the 250uL of inoculum (final volume 500uL per well).

19

Incubate at **37 °C** for ~ **65:00:00** .

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Day 5

20 

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

21 

Put about **500 µl formalin 4%** into each well on top of the overlay media.

22 

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

23 Remove **media** from the well plate.

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

25 

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.

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