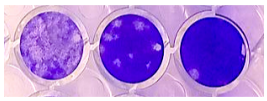
Apr 17, 2020

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



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Björn eyer[1]

[1]Institut Pasteur

Coronavirus Method De...

Bjoern Meyer
Institut Pasteur

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

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Abstract

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

Attachments

DOCX

SARSCoV2_SemiSolid_P...

277KB

Materials

MATERIALS

- Ethyl Alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E7023
- MEM **Thermo Fisher Catalog #**11095080
- UltraPure™ 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% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**HT501128-4L
- Crystal violet solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**HT90132
- VERO C1008 [Vero 76 clone E6 Vero E6] (ATCC® CRL-1586™) **ATCC Catalog #**CRL-1586

	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
	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 start

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 .

Note

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 .

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 7.5×10^4 **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).

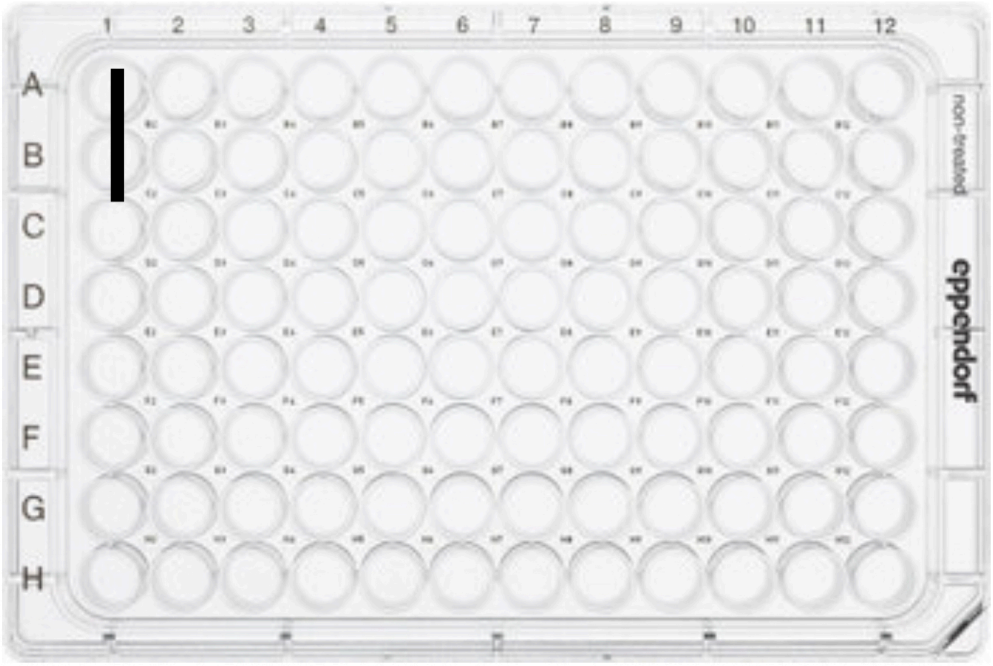
10 In each well, put 270 µL MEM (serum-free) .

11 Add 30 µL viral solution in the first row (A) and mix thoroughly.

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

12 Discard your tips.

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 .

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 00500 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.