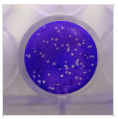

Mar 17, 2020

SARS-CoV-2 virus plaque assays [Biosafety Level 3]

tenOever Lab[1]

[1]Icahn School of Medicine at Mount Sinai

1 *Works for me* dx.doi.org/10.17504/protocols.io.bdtni6me

Coronavirus Method Development Community

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ABSTRACT

Method to plaque the SARS-CoV-2 virus

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Downloaded from: https://commons.wikimedia.org/wiki/File:Plaque_assay_macro.jpg

EXTERNAL LINK

https://bit.ly/3d6OUny

SAFETY WARNINGS

Biosafety Level 3

Media Recipes

1 Media recipe for modified DMEM: [DMEM with 2% FBS]:

- **475 ml** DMEM (w/ L-Glutamate, Sodium pyruvate)
- **5 ml** 100x P/S
- **5 ml** 100x Non-Essential Amino Acids (NEAA) Solution
- **5 ml** 1M HEPES
- **10 ml** FBS

Citation: tenOever Lab (03/17/2020). SARS-CoV-2 virus plaque assays [Biosafety Level 3]. https://dx.doi.org/10.17504/protocols.io.bdtni6me

2 Media recipe for 2XMEM: Sterile filter with 22 µm filtration unit:

- **100 ml** 10x MEM
- **10 ml** 100x P/S
- **10 ml** 100x L-Glutamine
- **6 ml** 35%BSA
- **340 ml** sterile ddH2O
- **10 ml** 1M HEPES
- **24 ml** 5% NaHCO3

3 Overlay Media (Ensure this is just warm to the touch when added to cells)

- **1 ml** PBS
- **31.5 ml** 2X MEM
- **17.5 ml** Oxoid Agar

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4 Seed Vero E6 cells in 6-well plates overnight at **37 °C** (10^6 cells/well)

5 Dilute the SARS-CoV-2 virus logarithmically in infection media

6 Replace media with modified DMEM and add diluted virus in each well (200 µl/well)

7 Allow virus adsorption for **01:00:00** , agitating the plate every **00:10:00** .

8 Heat the overlay media/oxoid agar mixture to ensure homogenous consistency and add it to each well (2ml/well), incubate at **37 °C** incubator for **72:00:00** .

9 Fix the plate with 5% formaldehyde (w/ methanol), (1.5 ml/per well) **Overnight** to ensure virus inactivation and Stain with crystal violet for **01:00:00** to determine viral titers (PFU/ml)

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