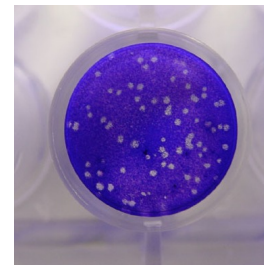


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SARS-CoV-2 virus plaque assays [Biosafety Level 3]

DOI

<https://dx.doi.org/10.17504/protocols.io.bdt6ni6me>



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Coronavirus Method De...



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Protocol status: Working

We use this protocol and it's working

Created: March 17, 2020

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Protocol Integer ID: 34382

Keywords: COVID19, SARS-CoV2, viral plaque assay, virus, sar, biosafety level,

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

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

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

SARS-CoV2 virus plaque assays (Biosafety level 3)

4 Seed Vero E6 cells in 6-well plates overnight at 37 °C (10⁶ 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)