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

tenOever Lab

1Icahn School of Medicine at Mount Sinai

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

This is an open access protocol distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

## ABSTRACT

Method to plaque the SARS-CoV-2 virus

Image attribution:
Y tambe / CC BY-SA (http://creativecommons.org/licenses/by-sa/3.0/)
Downloaded from: https://commons.wikimedia.org/wiki/File:Plaque_assay_macro.jpg

## EXTERNAL LINK


## DOI

dx.doi.org/10.17504/protocols.io.bdtni6me

## PROTOCOL CITATION

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

## KEYWORDS

COVID19, SARS-CoV2, viral plaque assay

## LICENSE

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

## CREATED

Mar 17, 2020

## LAST MODIFIED

Mar 17, 2020

## PROTOCOL INTEGER ID

34382

## 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 (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.

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

This is an open access protocol distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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).