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

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Coronavirus Method Development Community

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protocols.io

ABSTRACT

Method to plaque the SARS-CoV-2 virus

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SAFETY WARNINGS

Biosafety Level 3

External link:

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

Created: Mar 17, 2020
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

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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).