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

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Coronavirus Method Development Community

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ABSTRACT

Method to plaque the SARS-CoV-2 virus

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SAFETY WARNINGS

ABSTRACT
Method to plaque the SARS-CoV-2 virus

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 \( 37 \, ^\circ\text{C} \) (10^6 cells/well)

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

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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)