

SARS-CoV-2 Cytopathic Effect Assay

Target Type	Phenotypic
Cell Line (if applicable)	Vero E6 selected for high ACE2 expression (PMID: 17200104)
Readout	Luminescence
Direction of activity	Activation (rescue of cytopathic effect, CPE)
Controls used	Positive control: Cells without SARS-CoV-2; Calpain inhibitor IV (Millipore Sigma, Cat #: 208724) Negative control: DMSO

Assay Overview

A live SARS-CoV-2 virus assay measuring the ability of compounds to reverse the viral induced cytopathic effect (CPE) in Vero E6 host cells. The CPE reduction assay is a popular and widely used assay format to screen for antiviral agents because of its ease of use in quantitative high-throughput screening (qHTS). In this assay, viral infection and replication leads to a loss of host cell viability, which is indirectly measured by an endpoint assessment of host cell viability after 72 hr. The CPE reduction assay indirectly monitors the ability of compounds to inhibit viral replication and infection through various molecular mechanisms, including direct inhibition of viral entry or enzymatic processes as well as acting on host pathways that modulate viral replication. Compounds with antiviral activity protect the host cells from the CPE of the virus, thereby increasing viability.

Protocol

Vero E6 cells were premixed with SARS-CoV-2 for 5-10 min, then dispensed into assay ready plates (pre-dispensed with compounds and controls). Cells and virus were incubated with compounds for 72 hr, then viability was assayed by Vero E6 host cell ATP content.

Process	Notes
1 60 nL/well of compounds and positive control (Calpain inhibitor IV) acoustically dispensed into 384-well assay plates (Greiner, black clear bottom, tissue culture treated plates, #: 781091).	Calpain inhibitor IV (Millipore Sigma, Cat #: 208724) stock concentration 2 mg/mL (final assay concentration 4 µg/mL)
2 5 µl/well of media was dispensed into assay plates	MEM, 1% Pen/Strep/GlutaMax, 1% HEPES, 2% HI FBS
3 Dispensed 25 µl/well of Vero E6 cells inoculated with SARS CoV-2 (USA_WA1/2020) at multiplicity of infection (MOI) of 0.002 suspended in media. Final cell density was 4000 cells/well.	n/a
4 Incubate for 72 hr	37°C, 5% CO ₂ , 90% humidity
5 Dispense 30 µl/well of CellTiter-Glo	Promega, Cat #: G7573
6 Incubate at RT for 10 minutes	n/a
7 Read on CLARIOstar (BMG Labtech) or Envision (Perkin Elmer) multimode plate reader	Luminescence read

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Normalization/Assay interpretation

Data is normalized to the negative control (DMSO) and positive controls (cells without virus and Calpain inhibitor IV) for each plate. In the SARS-CoV2 CPE assay, an inactive compound yields a flat line, with 0% response; a compound that inhibits the viral induced cytopathic effect produces a curve that approaches 100% response.

