

क्षेत्रीय जैवप्रौद्योगिकी केन्द्र

राष्ट्रीय महत्ता की संस्था, जैवप्रौद्योगिकी विभाग,
भारत सरकार द्वारा यूनेस्को के तत्वावधान में स्थापित

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SARS-CoV2 Antiviral Testing Report

(To be used only for research purpose)

Assay Method – Cytotoxicity

- The assay is done in a 96-well plate format in 3 wells for each sample.
- 1×10^4 VeroE6 cells were plated per well and incubated at 37-degree C overnight for the monolayer formation.
- Next day, cells were incubated with the test substance (TS) at the indicated concentration. The cells without test substance was the control.
- 24 and 48 hours later, cells were stained with Hoechst 33342 and Sytox orange dye.
- Images were taken at 10X, 16 images per well, which covers 90% of well area using ImageXpress Microconfocal (Molecular Devices).
- Hoechst 33342 nucleic acid stain is a popular cell-permeant nuclear counterstain that emits blue fluorescence when bound to dsDNA. It stains all the live and dead cells.
- Sytox orange dye stains nucleic acids in cells with compromised membranes. This stain is an indicator of cell death.
- First, the software will count total number of cells in the Hoechst image.
- In the Sytox image, it will count, among Hoechst positive cells, how many cells are positive for Sytox.

Assay Method – Antiviral screening

- The assay is done in a 96-well plate format in 3 wells for each sample.
- 1×10^4 VeroE6 cells were plated per well and incubated at 37-degree C overnight for the monolayer formation.
- Next day, cells were incubated with the test substance (TS) at the indicated concentration. The cells without test substance was the control. The cells were infected with SARS-CoV2 at a MOI of 0.01.
- 24 and 48 hours later, viral RNA was extracted from 100 μ l culture supernatant and subjected to qRT-PCR (in duplicates) where Ct values for N and E gene sequence were determined.
- Inhibition of virus replication is determined based on the fold change in the Ct value in TS-treated cells compared to the control.
- Remdesivir was used as a positive control for viral inhibition.

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Results

| Compound | Concentration | % Cell viability | | % inhibition of virus replication | | | |
|------------|------------------------------|---------------------|---------------------|-----------------------------------|-------|---------------------|-------|
| | | 24 h post-treatment | 48 h post-treatment | 24 h post-infection | | 48 h post-infection | |
| | | | | E | N | E | N |
| Remdesivir | 10 μ M | 99.23 | 94.37 | 92.62 | 93.55 | 99.96 | 99.96 |
| Grolyfe | 10 μ g/ml concentration | 53.66 | 42.06 | 96.13 | 96.38 | 99.96 | 99.95 |
| Grolyfe | 1 μ g/ml concentration | 83.94 | 91.38 | 56.47 | 63.16 | 34.54 | 39.73 |
| Grolyfe | 0.1 μ g/ml concentration | 84.89 | 100.75 | - | - | - | - |

Nirpendra Singh
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