RGCB
DISCOVERIES FOR A BETTER TOMORROW

Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram 695014, Kerala State, India.
An Autonomous National Institute for Discovery, Innovation & Translation in Biotechnology and Disease Biology,
Government of India, Ministry of Science & Technology, Department of Biotechnology.

राजीव गाँधी जैव प्रौद्योगिकी केन्द्र, तिरुवनन्तपुरम 695 014, केरल, भारत
जैवप्रौद्योगिकी और रोग जीवविज्ञान में आविष्कार, नवीनता एवं अनुवाद की स्वायत्त राष्ट्रीय संस्थान,
भारत सरकार विज्ञान एवं प्रौद्योगिकी मंत्रालय, जैवप्रौद्योगिकी विभाग.

ANALYSIS REPORT

Date : 13-07-2021

REPORT NO: RGCB/CCL/MP/0010/16

Disclaimer:

This document is purely the test report of the samples provided to us and not a validation report. The client should not use this report as a quality certificate for marketing this product and RGCB does not hold any responsibility for doing the same.

PART 1:

Assay carried out at Central Cell line Repository of RGCB, Trivandrum

Name of the Test compound: Tablets

Supplied by : Zum Heilen Diagnostic and Therapeutics Pvt Ltd.

The dilution used 1:500; 1:1000 of the sample solution (20mg of the sample dissolved in water)

Cytotoxicity assay using chromatin condensation

The HEK293T cells stably expressing human ACE2 were grown on 96 glass bottom plates and allowed to grow for 24 hours. Then the cells were stained with fluorescent nuclear dye Hoechst 33342 at 5 ug per ml for five minutes. The cells were washed and replaced with fresh medium and maintained in CO_2 incubator at 37 ° C for 4 hours. The test samples were incubated with the cells at the indicated concentration. Fluorescent images were captured at 48h using DAPI filter set to visualize cell death using an inverted Fluorescent Microscope Nikon TiE. The images were captured with an EMCCD camera from Andor using NIS element software (Nikon). The cell death was interpreted based on the condensed chromatin compared to the control untreated wells (Figure).

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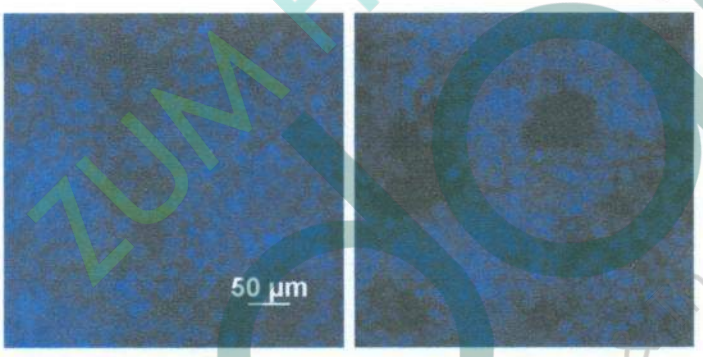

Control 1:500

Based on the cytotoxicity, the sample at 1:500 dilution showed no cytotoxicity to the HEK293T cells.

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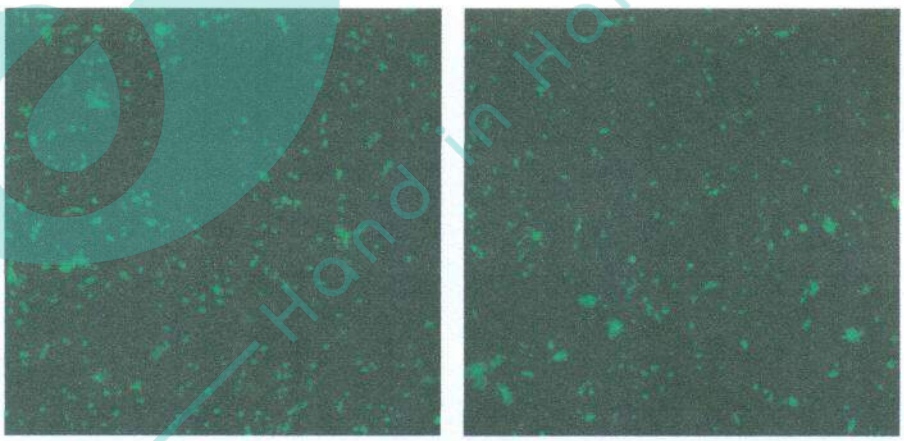
FINAL REPORT OF THE SARS CoV2 pseudovirion Assay

(To be used for Research)

Pseudovirion Assay:

The assay is based on the lentiviral backbone expressing Td tomato as a traceable marker. We have utilized stable colon cancer cell HEK293T expressing human ACE2 as the SARS permissive cells. The procedure involves transfection of HEK Lenti Cells (Invitrogen) with the expression vector encoding GFP, a plasmid expressing Spike, and plasmids expressing the minimal set of lentiviral proteins necessary to assemble viral particles (Gag/Pol,Rev). The cells were transfected with the expression vectors prepared via Quiagen Midi prep using lipofectamine 2000 as per the manufacturer's instruction. After 6h, the cells were replaced with fresh medium containing serum. From the transfected cells, SARS- CoV2- Spike-pseudotyped lentiviral particles were collected at 72 hours and filtered using 0.45 micron filter and used to infect the HEK293T– hACE2 cells using polybrene as per the standard protocol. The test samples were incubated with pseudovirions containing medium at indicated dilutions. The media diluted pseudovirion sample acts as the control. After 48h the cells were imaged under florescent microscope and cells expressing GFP fluorescence were counted and percentage positivity was calculated based on the total number of cells in the field.



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The given test compound (Tablets) shown very low inhibition (20% at 1:500 dilution) of SARS CoV2 pseudovirion entry to target cells.

SANTHIK SL

Research Fellow.

Dr.T.R.Santhosh Kumar

Scientist G

Dr. T. R. SANTHOSHKUMAR
Scientist G
Cancer Research
Rajiv Gandhi Centre for Biotechnology
Thiruvananthapuram, Kerala-695 014