REPORT OF ANALYSIS

Analysis of degradation activity of HCT Mouth Spray V2 against Recombinant Subunit 2 (S2) of Spike Glycoprotein of SARS-CoV-2

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Sample Description

Name/ ID	: HCT Mouth Spray V2
Received	: 11 August 2021
Type	: Liquid
Expiry Date	: Not mentioned
Container	: Plastic bottle
Storage	: Room temperature

RESULT : Degradation rate of S2 subunits by HCT Mouth Spray V2

	Volume (uL)	Degradation rate (%) per hour	Method*
HCT Mouth Spray V2	5	100	SDS-PAGE

*See Annex for detailed method

Remarks:

- ✓ The degradation rate is measured *in vitro* as detailed in the method. The rate may be different for measurement done under different conditions.
- ✓ The protein concentration of the sample is unknown. The result may be different for the sample with different protein concentrations.
- ✓ The degradation rate does not refer to the amount of degraded protein, rather it refers to the changes on the band thickness on the SDS-PAGE.
- ✓ The result does not reflect the ability of the sample to inhibit living SARS-CoV-2 virus.

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ANNEX

Method of analysis

Five (5) µl of HCT Mouth Spray V2 was mixed with 10 µl of recombinant S2 subunit of SARS-CoV-2 virus (RayBiotech Life, Inc., GA, USA). The final concentration of the recombinant S2 subunit was 0.5 mg/ml each. The cocktail was then incubated at 37°C for 2h. The reaction was stopped by the addition of 2X SDS-Sample buffer and heated at 100 °C for 10 min. The mixture was then loaded onto sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE), with 4% and 15% stacking and resolving gel, respectively. A low molecular weight protein marker (GE Healthcare) was also loaded as a size reference along with the sample. The separation of protein fragments inside the mixture was then performed under the constant current parameter at 37 mA until the dye reached the end of the gel. The gel was then stained using Coomassie Blue solution for 30 min, followed by the staining using acetic acid for another 30 min. To visualize the protein fragments on the gel, Bio-Rad Gel Doc was used. To measure the degradation rate, the surface area of S2 subunit bands on the gel were measured using ImageJ software (https://imagej.nih.gov/ij/download.html). The surface area at 0 h incubation was adjusted as 100%. The degradation rate was calculated based on the ratio of the reduction of surface area to the surface area at 0 h.