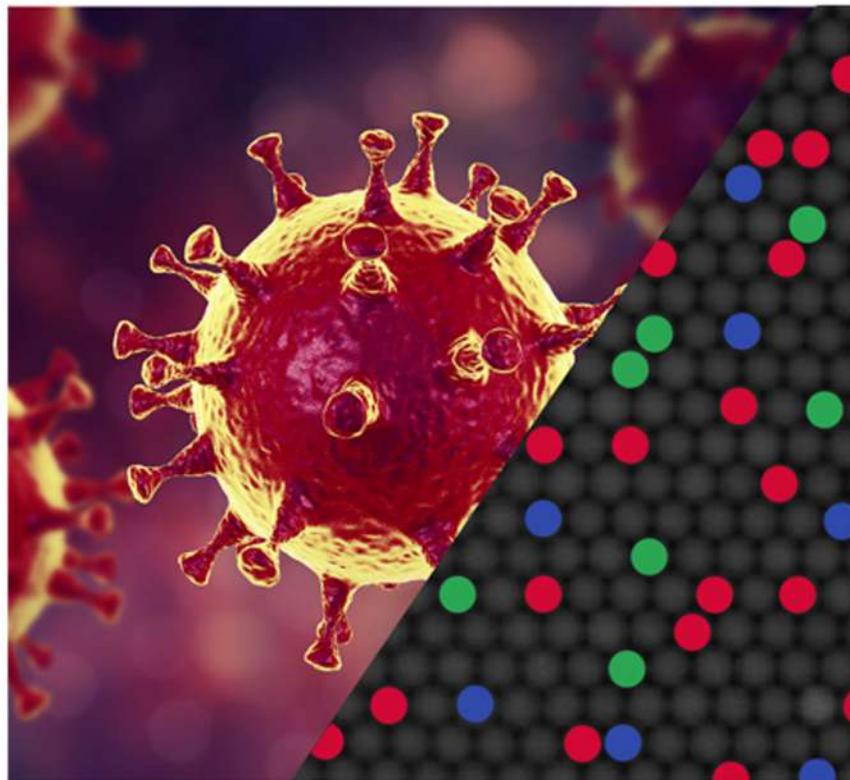




# A 3-color Crystal Digital PCR™ kit for detection of COVID-19



# Development of one-step RT-dPCR models for COVID-19 detection

The 2019-2020 outbreak of COVID-19 caused by the SARS-CoV-2 virus first reported in Wuhan, Hubei, China has been declared a pandemic by the World Health Organization. To facilitate the action of health authorities, the development of robust laboratory tests is of primary importance. Using the numerous publicly accessible SARS-CoV-2 and SARS-related sequences, several PCR-based assays specific for SARS-CoV-2 have been designed (Chan et al., 2020). The Naica™ compatible 3-color Crystal Digital PCR™ kit (Figure 1), developed by ApexBio (Hsin-chu Science-based Industrial Park) includes primers and FAM- and HEX-labeled probes specific to two distinct regions (Nucleocapside (N) and ORF1ab genes, respectively) of the SARS-CoV-2 positive strand RNA genome. The 3<sup>rd</sup> channel of the Naica platform has been used as an internal PCR reference detecting a human housekeeping gene with a Cy5-labeled probe. This single assay design permits the simultaneous detection of two independent SARS-CoV-2 sequences while concurrently monitoring PCR effectiveness using the third channel of detection (Figure 2).



Figure 1: The RUO ApexBio-developed ready-to-use kit contains all reagents required to perform a one-step RT 3-color Crystal Digital PCR on the Naica System.

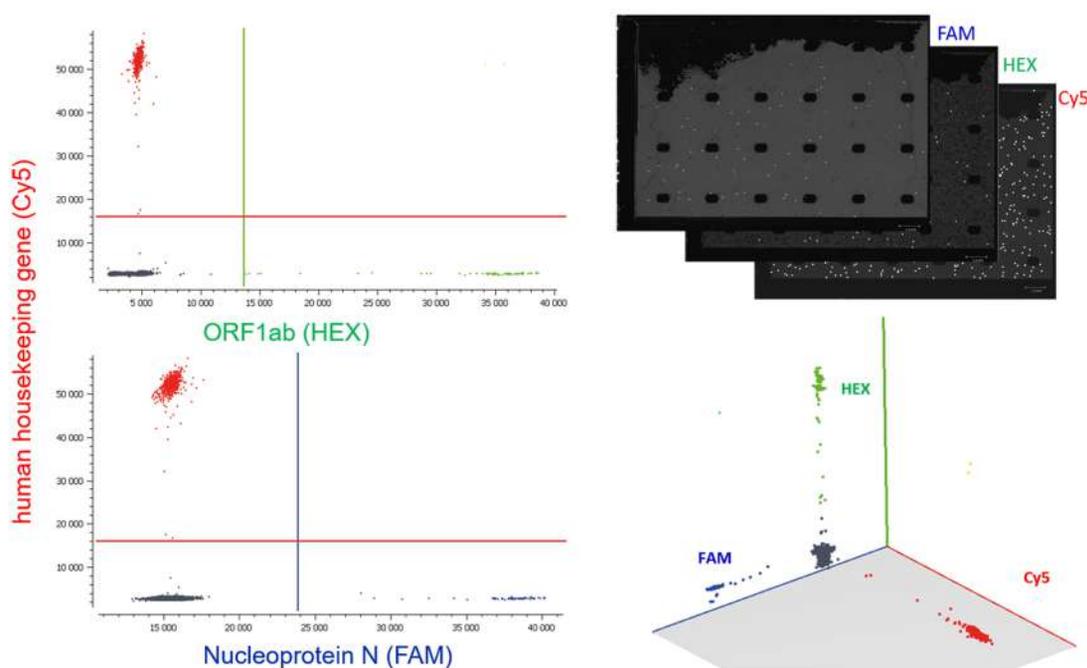


Figure 2: Crystal Miner-generated 2D (left) and 3D dot plots (right) and crystal droplet images obtained on positive controls containing human RNA and synthetic target sequences of the RUO RT-dPCR SARS-CoV-2 detection kit. FAM- and HEX-labeled probes specifically target two distinct regions (Nucleocapside (N) and ORF1ab genes, respectively) of the SARS-CoV-2 genome, whereas a Cy5-labeled probe detects a control human housekeeping gene. Thresholds are automatically set using the integrated Crystal Miner software.

## Sensitive and specific detection of COVID-19

An experimental model containing synthetic sequences targeted by the SARS-CoV-2 detection kit was serially diluted and seven dilution points were assessed in triplicate. A total of 1ng of human RNA was added to each replicate. The results indicated a robust and specific detection of SARS-CoV-2 sequences down to 0.6 copies per  $\mu\text{l}$  of positive control (5 copies per 25 $\mu\text{l}$  reaction) of the Nucleocapside (N) gene and down to 0.9 copies per  $\mu\text{l}$  of positive control (7 copies per 25 $\mu\text{l}$  reaction) of the ORF1ab gene in all tested samples. Further dilutions showed an extremely sensitive but stochastic detection down to 0.25 copies per  $\mu\text{l}$  of positive control (2 copies per 25 $\mu\text{l}$  reaction) for both genes (Figure 3). In parallel, a total of 15 controls containing only human RNA were tested as negative controls and no false positives were observed.

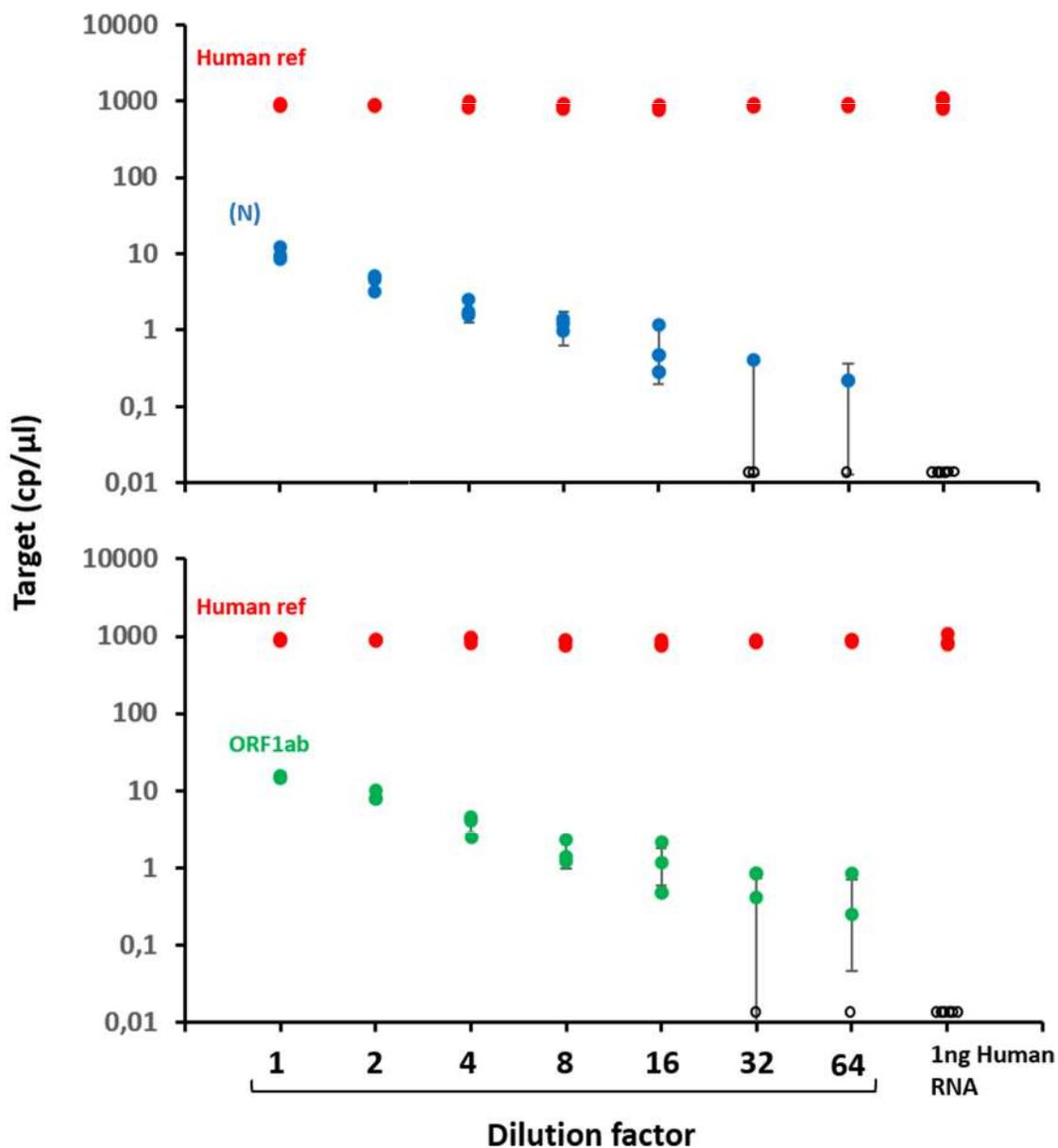


Figure 3: Sensitivity of the 3-color RUO RT Crystal Digital PCR kit for SARS-CoV-2 detection. Serial dilutions of SARS-CoV-2 synthetic targets were assayed in triplicate in a background of 1ng of human RNA. A total of 8 $\mu\text{l}$  of positive controls was added to each 25 $\mu\text{l}$  reaction. The vertical bars represent the theoretical 95% Poisson confidence intervals for the pool of 3 replicates. The empty circles represent replicates where SARS-CoV-2 sequences were not detected.

## Application Note Highlights

-  The SARS-CoV-2 detection kit is a ready-to-use RUO 3-color RT Crystal Digital PCR kit simultaneously quantifying two SARS-CoV-2 specific sequences and an internal human control in a single reaction
-  Robust and sensitive detection using the Naica platform was observed down to 0.6 copies per  $\mu\text{l}$  and 0.9 copies per  $\mu\text{l}$  of the SARS-CoV-2 Nucleocapside (N) and ORF1ab genes, respectively
-  Further dilutions showed an extremely sensitive but stochastic detection down to 0.25 copies per  $\mu\text{l}$  of the SARS-CoV-2 positive controls
-  No false positives were observed in 15 negative controls containing 1ng of human RNA per 25 $\mu\text{l}$  reaction

 To learn more about digital PCR, please visit Stilla Technologies' Learning Center at [www.gene-pi.com](http://www.gene-pi.com)

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