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 Apex-Bio (Hsinchu 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 3rd 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.

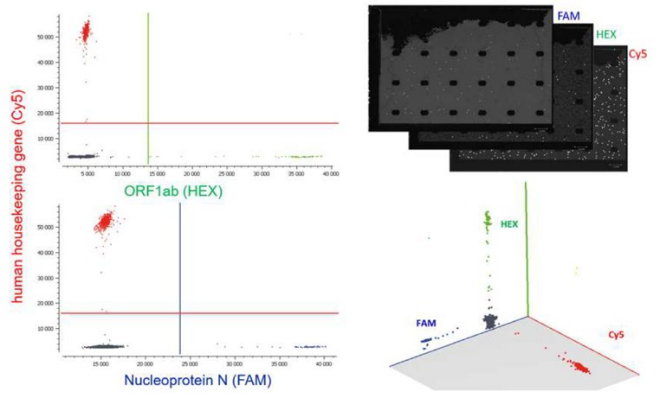

Figure 2: Crystal Miner-generated 2D (left) and 3D dot plots (right) and crystal droplet images obtained on positive controls containing human RNA and synthetic target sequences of the RUO RT-dPCR SARS-CoV-2 detection kit. FAM- and HEX-labeled probes specifically target two distinct regions (Nucleocapside (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 µl of positive control (5 copies per 25µl reaction) of the Nucleocapside (N) gene and down to 0.9 copies per µl of positive control (7 copies per 25µl reaction) of the ORF1ab gene in all tested samples. Further dilutions showed an extremely sensitive but stochastic detection down to 0.25 copies per µl of positive control (2 copies per 25µl 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.

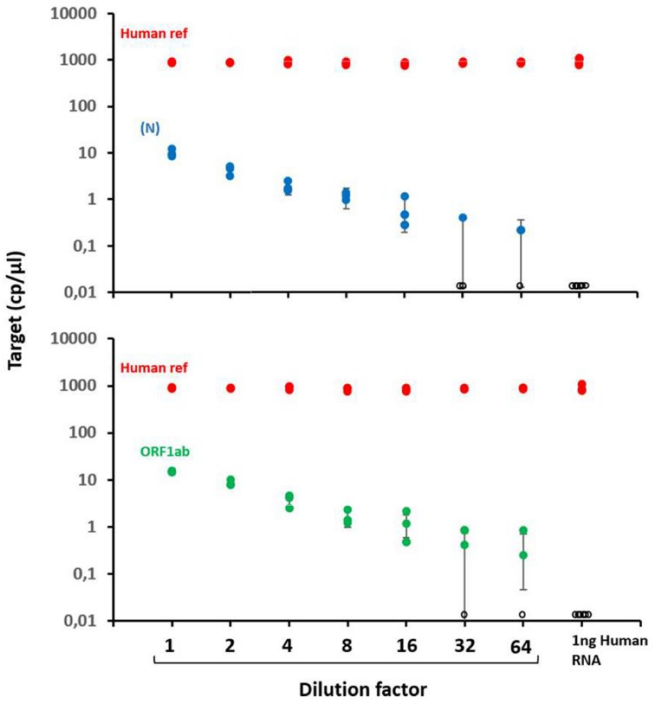

Figure 3: Sensitivity of the 3-color RUO RT Crystal Digital PCR™ kit for SARS-CoV-2 detection. Serial dilutions of SARS-CoV-2 synthetic targets were assayed in triplicate in a background of 1ng of human RNA. A total of 8µl of positive controls was added to each 25µl reaction. The vertical bars represent 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.

REFERENCES

Chan JF, Yip CC, To KK, Tang TH, Wong SC, Leung KH, Fung AY, Ng AC, Zou Z, Tsoi HW, Choi GK, Tam AR, Cheng VC, Chan KH, Tsang OT, Yuen KY. Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-polymerase chain reaction assay validated in vitro and with clinical specimens. J Clin Microbiol. 2020 Mar 4.

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 µl and 0.9 copies per µl 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 µl of the SARS-CoV-2 positive controls
- No false positives were observed in 15 negative controls containing 1ng of human RNA per 25µl reaction

To learn more about digital PCR, please visit Stilla Technologies' Learning Center at **stillatechnologies.com/digital-pcr**