# ViroKey™ SARS-CoV-2 RT-PCR Test

## Instructions for Use

Version 1.1

**For use under an Emergency Use Authorization Only**

**For Prescription Use Only**

IVD

Rx Only

Vela Operations Singapore Pte Ltd, #05-07 The Kendall,
50 Science Park Road, Singapore 117406

REF 300681

Σ 4x50 tests

MAT PS102315E

Consult instructions for use

# Contents

## Kit contents

| Kit item no. | Item | Cap color | Description | Quantity | Volume / tube |
|---|---|---|---|---|---|
| 300681 | SARS-CoV-2 M1-1 | Green | Mix 1-1 | 4 | 60 µL |
| | SARS-CoV-2 M1-2 | Green | Mix 1-2 | 4 | 60 µL |
| | RNA4 M2 | Orange | Mix 2 | 8 | 700 µL |
| | RNA4 M3 | Pink | Mix 3 | 4 | 240 µL |
| | NC5 | Yellow | Negative control (NC) | 4 | 600 µL |
| | SARS-CoV-2 PC | Blue | Positive control (PC) | 4 | 600 µL |
| | EC8 | Red | Extraction control (EC) | 4 | 600 µL |

## Symbols

Contains reagents sufficient for <N> tests


Expiration date

*In vitro* diagnostic medical devices

Prescription device

Catalog number

Component

Number

Content

Lot number

Control

Negative control

Positive control

Document / label identification number


Temperature limitations


Legal manufacturer


Refer to instructions for use

## Storage

The components of the ViroKey™ SARS-CoV-2 RT-PCR Test should be stored at −20°C ± 5°C and are stable until the expiration date stated on the label. RNA4 M3 is an enzyme, which is in liquid state. Except RNA4 M3, all reagents should be thawed completely before use.

| Kit item no. | Item | Quantity | Shipping Condition | Storage Condition |
|---|---|---|---|---|
| 300681 | SARS-CoV-2 M1-1 | 4 | Dry ice | -25°C – -15°C |
| | SARS-CoV-2 M1-2 | 4 | Dry ice | -25°C – -15°C |
| | RNA4 M2 | 8 | Dry ice | -25°C – -15°C |
| | RNA4 M3 | 4 | Dry ice | -25°C – -15°C |
| | NC5 | 4 | Dry ice | -25°C – -15°C |
| | SARS-CoV-2 PC | 4 | Dry ice | -25°C – -15°C |
| | EC8 | 4 | Dry ice | -25°C – -15°C |

## Intended use

ViroKey™ SARS-CoV-2 RT-PCR Test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, oropharyngeal swab specimens and nasopharyngeal wash/aspirate or nasal aspirate specimens) collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-Cov-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The ViroKey™ SARS-CoV-2 RT-PCR Test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The ViroKey™ SARS-CoV-2 RT-PCR Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

## Warnings and Precautions

- This test is for use under an Emergency Use Authorization.
- For Prescription Use Only.
- For *in vitro* diagnostic use only (IVD).
- The ViroKey™ SARS-CoV-2 RT-PCR Test has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. 263a, that meet requirements to perform high complexity tests.
- The ViroKey™ SARS-CoV-2 RT-PCR Test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens. The ViroKey™ SARS-CoV-2 RT-PCR Test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- The product is to be used by qualified and trained clinical laboratory personnel only.
- Strict compliance with the instructions for use is required for optimal PCR results.
- Each tube of reagent is designed for 50 reactions.
- Do not use expired kit components. Expiration dates are printed on the box and labels of all components. RNA4 M3 is an enzyme, which is in liquid state. Except RNA4 M3, the rest of the ViroKey™ SARS-CoV-2 RT-PCR Test components should be thawed completely at room temperature (approximately 15°C – 25°C) for up to 30 minutes before use.
- RNA4 M3 should be used directly out of the freezer or kept on ice when performing reagent preparation. Handle carefully to avoid contamination and store the remaining RNA4 M3 immediately after use at ≤ −20°C for subsequent reactions.
- All reagents, except RNA4 M2 and RNA4 M3, require thorough mixing by quick vortex. Mix RNA4 M2 and RNA4 M3 by gentle inversion. Centrifuge all tubes briefly to collect the contents at the bottom of the tubes. Avoid foaming of the reagents.
- All relevant documents (refer to “Resources” section) should be read thoroughly before performing the assay.
- Mutations that arise within the highly conserved regions of the viral genome covered by the kit’s primers and / or probes may result in failure to detect the presence of the virus.
- May cause allergic skin reactions.
- May be harmful if swallowed.
- Use personal protective equipment as required.
- For additional information, please refer to the Material Safety Data Sheet (MSDS).

- All samples and waste should be considered potentially infectious. Clean and disinfect all work surfaces thoroughly with disinfectants recommended by local authorities.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection when handling samples and kit reagents.
- Clean and decontaminate work area and instruments, including pipettes, with commercially available decontamination products.
- Avoid microbial and nuclease contamination of reagents when removing aliquots from reagent bottles. Use sterile disposable pipette tips.
- To avoid environmental contamination by amplicons, do not remove the PCR seal after amplification.
- Wash hands thoroughly after handling biological samples and kit reagents.

## Safety information

- When working with samples and chemicals, always wear a suitable lab coat, disposable gloves, protective goggles, respirator masks (if required) and surgical mask. For more information on the *Sentosa*® SX Virus Total Nucleic Acid Kit v2.0 (4x24) and the ViroKey™ SARS-CoV-2 RT-PCR Test, please refer to the respective material safety data sheets (MSDSs).
- For more safety information on the instruments, please refer to the relevant instrument user manual.
- Discard samples and waste according to local safety regulations.

## Quality control

In accordance with Vela Diagnostics' ISO 13485-certified Quality Management System, each lot of the ViroKey™ SARS-CoV-2 RT-PCR Test is tested against predetermined specifications to ensure consistent product quality.

## Introduction

The ViroKey™ SARS-CoV-2 RT-PCR Test comprises a ready-to-use system for the detection of SARS-CoV-2 RNA by Reverse Transcription PCR (RT-PCR) on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System or the *Sentosa*® SA201 Real-Time PCR Instrument (hereinafter known as *Sentosa*® SA201), with manual nucleic acid extraction and Reverse Transcription PCR assay set-up. The ViroKey™ SARS-CoV-2 RT-PCR Test is an assay containing four primers and two probes that target the *ORF1a* and *RdRp* genes of SARS-CoV-2 for direct detection of the specific amplicons in two separate RT-PCR reactions. The genes are detected in the green fluorescence channel on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System or *Sentosa*® SA201.

In addition, the ViroKey™ SARS-CoV-2 RT-PCR Test contains a third set of primers / probes designed to detect an extraction control (EC) target in the red fluorescence channel. This extraction control can be used as a control for the nucleic acid extraction procedure and as a PCR inhibition control. The EC amplification system does not compromise the detection limit of the analytical SARS-CoV-2 PCR. The test also contains a negative control (NC) and a positive control (SARS-CoV-2 PC) that allows the user to assess whether the Reverse Transcription PCR reaction has been performed correctly.

## Principle

ViroKey™ SARS-CoV-2 RT-PCR Test uses TaqMan® probe chemistry for real-time RT-PCR detection of viral nucleic acid extracted from upper respiratory specimens using the *Sentosa*® SX Virus Total Nucleic Acid Kit (4x24) v2.0. The product includes two sets of oligonucleotide primer and probe mixtures (PPM) for the detection of either *RdRp* (tube M1-1) or *ORF1a* (tube M1-2) sequences from SARS-CoV-2 viral RNA with FAM reporter dye in the Green fluorescence channel. An extraction control (EC) consisting of a non-human synthetic DNA fragment is multiplex with each PPM using another reporter dye in the Red fluorescence channel to consolidate the reaction into a single well.

Additional materials in the ViroKey™ SARS-CoV-2 RT-PCR Test include enzyme mixes (tubes M2 and M3), Negative Control (tube NC), Positive control (tube PC) and extraction control (tube EC) to be added to each sample.

## Pathogen information

Coronaviruses, which are RNA viruses from the *Coronaviridae* family that are part of the *Coronavirinae* subfamily, cause infectious diseases that mainly infect the respiratory tract, resulting in respiratory tract infections (e.g., common cold) in humans[1]. Other symptoms include rhinitis, cough, sore throat, and fever[2].

Previously, six coronaviruses that can infect humans were identified – HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV)[3]. In December 2019, a novel coronavirus was discovered in Wuhan, China. The

newly discovered coronavirus is the causative agent of the COVID-19 disease. Human-to- human transmission of the virus via respiratory droplets has been confirmed[4]. In February 2020, the International Committee on Taxonomy of Viruses named the novel coronavirus Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2)[5].

## Limitations

- All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the assay independently.
- The ViroKey™ SARS-CoV-2 RT-PCR test does not include an Internal Control for RNA extraction/recovery. A known SARS-CoV-2 positive sample should be tested with every batch of patient specimens to monitor the integrity of these process steps.
- The ViroKey™ SARS-CoV-2 RT-PCR Test is validated for use on specimens obtained from nasopharyngeal swabs.
- The performance with upper respiratory specimens nasal self-collected under supervision of, or healthcare provider collected and mid-turbinate swabs, nasopharyngeal wash/aspirate or nasal aspirate specimens have not been evaluated.
- The interference of homeopathic medication has been observed with the ViroKey™ SARS-CoV-2 RT-PCR Test. Prospan® cough syrup interfered with the assay when tested at 10% (v/v) but not 5% (v/v).
- The ViroKey™ SARS-CoV-2 RT-PCR Test have not been evaluated for patients receiving intranasally administered influenza vaccine
- The ViroKey™ SARS-CoV-2 RT-PCR Test may cross react with SARS-CoV.
- Negative results do not preclude SARS-CoV-2 virus infection and should not be used as the sole basis for treatment or other patient management decisions.
- A false negative result may occur if a specimen is improperly collected, transported, or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- Positive and negative predictive values are highly dependent on prevalence rates. Positive test results are more likely to represent false positive results during periods of little/no SARS-CoV-2 activity when disease prevalence is low. False negative test results are more likely when prevalence of disease caused by SARS-CoV-2 is high.
- Do not use any reagent past the expiration date, as this may affect performance of the assay.

- Optimum specimen types and timing for peak viral levels during infections caused by a SARS-CoV-2 virus have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the viruses.
- If SARS-CoV-2 virus mutates in the rRT-PCR target region, the specific novel virus may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result. An interference study evaluating the effect of common cold medications was performed.
- The potential for the epidemiology and pathology of disease caused by a specific novel SARS-CoV-2 virus to affect test performance is not fully known. For example, clinicians and laboratories may not know the optimum types of specimens to collect, and when during the course of infection these specimens are most likely to contain levels of virus that can be readily detected.
- Detection of viral RNA may not indicate the presence of infectious virus or that SARS-CoV-2 viruses are the causative agent for clinical symptoms.
- The performance of this assay has not been established for screening of blood or blood products for the presence of SARS-CoV-2.
- This assay cannot rule out diseases caused by other bacterial or viral pathogens.

## Conditions of Authorization for the Laboratory

- The ViroKey™ SARS-CoV-2 RT-PCR Test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and other authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use- authorizations-medical-devices/vitro-diagnostics-euas.
- However, to assist clinical laboratories using the ViroKey™ SARS-CoV-2 RT-PCR Test ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

  (A) Authorized laboratories[1] using your product will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

  (B) Authorized laboratories using your product will use your product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not authorized under this EUA.

  (C) Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

(D) Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

(E) Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and to Vela Diagnostics USA, Inc. through email: support.us@veladx.com or at 877.593.7528 (in the U.S.) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

(F) Authorized laboratories will report adverse events, including problems with test performance or results, to MedWatch by submitting the online FDA Form 3500 (https://www.accessdata.fda.gov/scripts/medwatch/index.cfm?action=reporting.home) or by calling 1- 800-FDA-1088.

(G) All laboratory personnel using your product must be appropriately trained in PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use your product in accordance with the authorized labeling.

(H) Vela Diagnostics, authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

## Controls

A tube of Negative Control (NC), which is nucleic acid-free water, is included in the kit.

A tube of Positive Control (PC), consisting of an IVT-RNA fragment that can be amplified by both sets of primer/probes for Orf1a and RdRp gene, is included in the kit.

An Extraction Control (EC) is spiked into every test sample in every run and is needed to assess the efficacy of the nucleic acid extraction process, as well as test for the presence of inhibitors and validity of a negative result.

The ViroKey™ SARS-CoV-2 RT-PCR test does not include an Internal Control for RNA extraction/recovery. A known SARS-CoV-2 positive sample should be tested with every run of patients' specimens to monitor the integrity of these process steps.

## Results Interpretation

*Negativity / positivity*

The $C_T$ range to define negativity / positivity for negative control, positive control and sample is listed in the table below. If $C_T$ falls within the range, it is defined as positive (+); if $C_T$ falls out of the range or no $C_T$ is obtained, it is defined as negative (-). The green fluorescence channel detects the *ORF1a* and *RdRp* genes while the red fluorescence channel detects the extraction control.

| Fluorescence channel | $C_t$ range for negativity (-) and positivity (+) | | | | | |
|---|---|---|---|---|---|---|
| | Negative control | | Positive control | | Samples | |
| | - | + | - | + | - | + |
| Green M1-2 (SARS-CoV-2 Orfla) | ≥ 40.0 or Undet or no $C_t$ | < 40.0 | < 24.0, > 30.0 or no $C_t$ | 24.0 – 30.0 | > 40.0, < 10.0 or no $C_t$ | 10.0 – 40.0 |
| Green M1-1 (SARS-CoV-2 RdRp) | ≥ 40.0 or no $C_t$ | < 40.0 | < 24.0, > 30.0 or no $C_t$ | 24.0 – 30.0 | > 40.0, < 10.0 or no $C_t$ | 10.0 – 40.0 |
| Red (EC) | < 23.0, > 29.0 or no $C_t$ | 23.0 – 29.0 | < 23.0, > 29.0 or no $C_t$ | 23.0 – 29.0 | < 20.0, > 40.0 or no $C_t$ | 20.0 – 40.0 |

Note: Ct range updated after verification and validation. No $C_t$ is equivalent to Undet: Undetermined.

*Run validity*

Please refer to the table below for run validity interpretation.

| Control | SARS-CoV-2 M1-1 (Green) | SARS-CoV-2 M1-2 (Green) | Red* | Interpretation |
|---|---|---|---|---|
| Negative control | - | - | + | Run valid (proceed to PC) |
| | + | - | +/- | Run invalid. Repeat run. |
| | - | + | +/- | |
| | + | + | +/- | |
| | - | - | - | |
| Positive control | + | + | +/- | Run valid (proceed to positive sample control) |
| | + | - | +/- | Run invalid. Repeat run. |
| | - | + | +/- | |
| | - | - | +/- | |
| SARS-CoV-2 positive sample control | + | + | +/- | Run valid (proceed to result interpretation of samples) |
| | + | - | +/- | Run invalid. Repeat run. |
| | - | + | +/- | |
| | - | - | +/- | |

*Result interpretation of samples*

Please refer to the table below for result analysis. All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted

| SARS-CoV-2 M1-1 (Green) | SARS-CoV-2 M1-2 (Green) | Red* | Interpretation |
|---|---|---|---|
| + | + | M1-1: +/-<br>M1-2: +/- | All Target Results are valid. Result for SARS-CoV-2 RNA is Detected. |
| - | + | M1-1: +<br>M1-2: +/- | All Target Results are valid.<br>Result for SARS-CoV-2 RNA is Detected. |
| + | - | M1-1: +/-<br>M1-2: + | All Target Results are valid.<br>Result for SARS-CoV-2 RNA is Detected. |
| - | - | M1-1: +<br>M1-2: + | All Target Results are valid.<br>Result for SARS-CoV-2 RNA is Not Detected. |
| - | + | M1-1: -<br>M1-2: +/- | M1-1 Target Result is invalid.<br>Result for SARS-CoV-2 RNA is Detected. |
| + | - | M1-1: +/-<br>M1-2: - | M1-2 Target Result is invalid.<br>Result for SARS-CoV-2 RNA is Detected. |
| - | - | M1-1: +<br>M1-2: - | M1-2 Target Result is invalid.<br>Result inconclusive. Sample should be retested. If the result is still invalid, a new specimen should be obtained. |
| - | - | M1-1: -<br>M1-2: + | M1-1 Target Result is invalid.<br>Result inconclusive. Sample should be retested. If the result is still invalid, a new specimen should be obtained. |
| - | - | M1-1: -<br>M1-2: - | All Target Results are invalid.<br>Sample should be retested. If the result is still invalid, a new specimen should be obtained. |

*For positive samples, the fluorescence channel Cycling Red may be negative due to competition with the target channels.

**Run**: Whole run on the MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode
**Test**: Test to which the NC / PC belongs
**Sample**: Single sample in one well of the MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode

# Performance characteristics

## Analytical sensitivity

The analytical limit of detection (LoD) was assessed by the *Sentosa*® workflow (refer to **Flowchart 1**) for the ViroKey™ SARS-CoV-2 RT-PCR Test using inactivated SARS-CoV-2 (BEI, cat.# NR-52286). Serial dilutions of the SARS-CoV-2 in nasopharyngeal matrix were tested to determine the assay LoD. The preliminary LoD was determined by testing three replicates of inactivated virus dilutions between 1 x $10^5$ and 1 x $10^3$. The LoD was confirmed by testing at least 20 replicates. If the confirmatory study achieved a positivity of 100%, then a lower concentration was tested (with 20 replicates) until less than 100% positivity is obtained. The LoD was the lowest dilution giving a final sample detection ≥ 95% (**Table 1**). The overall LoD of the ViroKey™ SARS-CoV-2 RT-PCR Test is 560 GE/mL.

**Table 1. Limit of detection of the ViroKey™ SARS-CoV-2 RT-PCR Test.**

| Target channel | LoD with sample extraction (genomic equivalent/mL) | Detection % | Mean Ct ± SD |
|---|---|---|---|
| Green M1-1 (RdRP) | 560 | 100% (20/20) | 30.45 ± 0.77 |
| Green M1-2 (ORF1a) | 250 | 100% (20/20) | 34.18 ± 1.70 |

## Analytical reactivity and specificity

The analytical reactivity and specificity of the ViroKey™ SARS-CoV-2 RT-PCR Test are ensured by the selection of primers, probes and stringent reaction conditions.

### *Analytical reactivity*

To evaluate the analytical reactivity (inclusivity) of the ViroKey™ SARS-CoV-2 RT-PCR Test for SARS-CoV-2, *in silico* analysis was performed on all sequences available in the National Center for Biotechnology Information (NCBI) GenBank and Global Initiative on Sharing All Influenza Data (GISAID) databases. 27743 sequences (2351 from GenBank and 25212 from GISAID – sequences downloaded on May, 18 2020) were aligned against ViroKey™ SARS-CoV-2 RT-PCR Test primers and probes. The sequences were aligned with MAFFT (https://mafft.cbrc.jp/alignment/server/). *In silico* analysis concluded that ViroKey™ SARS-CoV-2 RT-PCR Test will detect all analyzed SARS-CoV-2 sequences in the NCBI GenBank (n=2351) and in GISAID (n=25212) databases. ViroKey™ SARS-CoV-2 RT-PCR Test *RdRp* primers and probes detected all sequences from the GenBank database with a maximum of 1 mismatch (10 sequences) and 100% of all sequences obtained from the GISAID database with a maximum of 1 mismatch (182 sequences). The ViroKey™ SARS-CoV-2 RT-PCR Test *ORF1a* primers and probes detected all sequences from the GenBank database with a maximum of 1 mismatch (9 sequences) and 100% of all sequences obtained from the GISAID database with a maximum of 1 mismatch (120 sequences). None of the mismatching sequences showed mismatches with the other target, therefore the inclusivity of the

assay is not expected to be affected.

***Analytical specificity (in silico)***

To evaluate the analytical specificity (cross-reactivity) of the ViroKey™ SARS-CoV-2 RT-PCR Test, *in silico* analysis of 64 bacterial/viral/fungal strains was performed. NCBI BLAST tool was used to check for cross-reactivity of the different primers and probes of the ViroKey™ SARS-CoV-2 RT-PCR Test against the non-redundant nucleotide database. BLAST tool search default parameters were used except for the "organism." The search was limited to using the taxonomy ID (taxid/txid) of the respective pathogen. Each primer and probe were compared against all available genome sequences of a certain taxid. Analyzed organisms are listed in Table 2.

**Table 2. *In silico* analysis for ViroKey™ SARS-CoV-2 RT-PCR Test.**

| | | ***In silico* analysis for % identity/homology** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Primers-Probe Mix 1 (M1-1)** | | | **Primers-Probe Mix 2 (M1-2)** | | |
| **Microorganism** | **Genbank Accession No.** | **Forward Primer** | **Reverse Primer** | **Probe** | **Forward Primer** | **Reverse Primer** | **Probe** |
| Coronavirus 229E | NC_002645.1 | No alignment was found | | | No alignment was found | | |
| Coronavirus OC43 | NC_006213.1 | **85%** | NA | NA | No alignment was found | | |
| Coronavirus HKU-1 | NC_006577.2 | **89%** | NA | NA | No alignment was found | | |
| Coronavirus NL63 | NC_005831.2 | No alignment was found | | | No alignment was found | | |
| SARS-coronavirus | NC_004718.3 | **89%** | 68% | **88%** | No alignment was found | | |
| MERS-coronavirus | NC_019843.3 | No alignment was found | | | No alignment was found | | |
| Human adenovirus 2 | AC_000007.1 | No alignment was found | | | No alignment was found | | |
| Human adenovirus 5 | AC_000008.1 | No alignment was found | | | No alignment was found | | |
| Human adenovirus 54 | NC_012959.1 | NA | 52% | NA | No alignment was found | | |
| Human adenovirus A | NC_001460.1 | No alignment was found | | | No alignment was found | | |
| Human adenovirus B1 | NC_011203.1 | No alignment was found | | | No alignment was found | | |
| Human adenovirus B2 | NC_011202.1 | No alignment was found | | | No alignment was found | | |
| Human adenovirus C | NC_001405.1 | No alignment was found | | | No alignment was found | | |
| Human adenovirus D | NC_010956.1 | No alignment was found | | | No alignment was found | | |
| Human adenovirus E | NC_003266.2 | No alignment was found | | | No alignment was found | | |
| Human adenovirus F | NC_001454.1 | No alignment was found | | | No alignment was found | | |
| Human adenovirus type 1 | AC_000017.1 | No alignment was found | | | No alignment was found | | |
| Human adenovirus type 35 | AC_000019.1 | No alignment was found | | | No alignment was found | | |
| Human adenovirus type 7 | AC_000018.1 | No alignment was found | | | No alignment was found | | |
| Human metapneumovirus | NC_039199.1 | No alignment was found | | | No alignment was found | | |

| | | *In silico* analysis for % identity/homology | | | | | |
|---|---|---|---|---|---|---|---|
| | | Primers-Probe Mix 1 (M1-1) | | | Primers-Probe Mix 2 (M1-2) | | |
| **Microorganism** | **Genbank Accession No.** | **Forward Primer** | **Reverse Primer** | **Probe** | **Forward Primer** | **Reverse Primer** | **Probe** |
| Human parainfluenza virus 1 | NC_003461.1 | No alignment was found | | | No alignment was found | | |
| Human parainfluenza virus 2 | NC_003443.1 | No alignment was found | | | No alignment was found | | |
| Human parainfluenza virus 3 | NC_001796.2 | No alignment was found | | | No alignment was found | | |
| Human parainfluenza virus 4a | NC_021928.1 | No alignment was found | | | No alignment was found | | |
| Influenza A virus H1N1 | GCF_000865725 | No alignment was found | | | No alignment was found | | |
| Influenza A virus H3N2 | GCF_000865085 | No alignment was found | | | No alignment was found | | |
| Influenza A virus H5N1 | GCF_000864105 | No alignment was found | | | No alignment was found | | |
| Influenza A virus H7N9 | GCF_000928555 | No alignment was found | | | No alignment was found | | |
| Influenza B virus | GCF_000820495 | No alignment was found | | | No alignment was found | | |
| Influenza C virus | GCF_000856665.10 | No alignment was found | | | No alignment was found | | |
| Human Parechovirus | NC_001897.1 | No alignment was found | | | No alignment was found | | |
| Enterovirus (e.g. EV68) | NC_038308.1 | No alignment was found | | | No alignment was found | | |
| Human respiratory syncytial virus | NC_001781.1 | No alignment was found | | | No alignment was found | | |
| Human rhinovirus 1 | NC_038311.1 | No alignment was found | | | No alignment was found | | |
| Human rhinovirus 3 | NC_038312.1 | No alignment was found | | | No alignment was found | | |
| Human rhinovirus 14 | NC_001490.1 | No alignment was found | | | No alignment was found | | |
| Human rhinovirus 89 | NC_001617.1 | No alignment was found | | | No alignment was found | | |
| Human rhinovirus C | NC_009996.1 | No alignment was found | | | No alignment was found | | |
| *Chlamydophila pneumoniae* | NC_002180.1 | No alignment was found | | | No alignment was found | | |
| *Haemophilus influenzae* | NZ_LN831035.1 | 48% | NA | NA | NA | NA | 60% |
| *Legionella pneumophila* | NZ_LR134380.1 | 48% | NA | 52% | 67% | NA | 56% |
| *Mycobacterium tuberculosis* | NC_000962.3 | NA | 52% | NA | 67% | NA | NA |
| *Streptococcus pneumoniae* | NZ_LN831051.1 | 48% | NA | 56% | NA | NA | 52% |
| *Streptococcus pyogenes* | NC_002737.2 | 48% | 56% | 64% | NA | NA | 60% |
| *Bordetella pertussis* | NC_018518.1 | NA | NA | 52% | 62% | NA | NA |
| *Mycoplasma pneumoniae* | NZ_CP010546.1 | No alignment was found | | | NA | NA | 52% |

| | | *In silico* analysis for % identity/homology | | | | | |
|---|---|---|---|---|---|---|---|
| | | Primers-Probe Mix 1 (M1-1) | | | Primers-Probe Mix 2 (M1-2) | | |
| **Microorganism** | **Genbank Accession No.** | **Forward Primer** | **Reverse Primer** | **Probe** | **Forward Primer** | **Reverse Primer** | **Probe** |
| *Pneumocystis jirovecii (PJP)* | GCF_001477535.1 | 56% | 68% | 52% | NA | NA | 52% |
| *Candida albicans* | GCF_000182965.3 | 63% | 56% | 60% | 62% | NA | 60% |
| *Pseudomonas aeruginosa* | GCF_000006765.1 | No alignment was found | | | 67% | NA | 52% |
| *Staphylococcus epidermis* | GCF_000007645.1 | 48% | 72% | NA | NA | 71% | NA |
| *Staphylococcus salivarius* | CP013216.1 | No alignment was found | | | No alignment was found | | |
| *Leptospira borgpetersenii* | GCF_000013945 | 48% | NA | NA | NA | NA | 56% |
| *Leptospira interrogans* | GCF_000092565 | 52% | NA | NA | No alignment was found | | |
| *Leptospira santarosai* | GCF_000313175 | No alignment was found | | | NA | 62% | NA |
| *Chlamydia psittaci* | NC_017287.1 | NA | NA | 56% | No alignment was found | | |
| *Coxiella burnetii (Q-Fever)* | NC_002971.4 | No alignment was found | | | 62% | NA | 60% |
| *Staphylococcus aureus* | NC_007795.1 | 48% | 68% | NA | No alignment was found | | |
| *Klebsiella pneumoniae* | GCF_000240185.1 | 52% | 52% | 56% | 62% | NA | 52% |
| *Corynebacterium diphtheriae* | NZ_LN831026.1 | 48% | NA | NA | NA | NA | 56% |
| *Legionella longbeachae** | GCF_000091785.1 | NA | 56% | 52% | **81%** | 67% | 56% |
| *Bacillus anthracosis (Anthrax)* | GCF_000008445.1 | 52% | 56% | NA | NA | NA | 56% |
| *Moraxella catarrhalis* | NC_014147.1 | NA | 60% | 56% | NA | 62% | 52% |
| *Neisseria elongata* | NZ_CP007726.1 | NA | NA | 52% | 62% | NA | NA |
| *Neisseria meningitidis* | NZ_LR134525.1 | NA | 52% | NA | 62% | NA | 52% |
| Pooled human nasal wash - to represent diverse microbial flora in the human respiratory tract | ZLYEM2C (HMP) | 74% | 64% | 64% | **86%** | 71% | 60% |
| Bat coronavirus HKU4-1, complete genome | NC_009019.1 | 37% | 48% | 40% | 48% | NA | 40% |
| Bat coronavirus HKU5-1, complete genome | NC_009020.1 | 37% | 56% | 44% | NA | 48% | NA |
| Bat coronavirus HKU9-1, complete genome | NC_009021.1 | 52% | 48% | 40% | NA | 48% | NA |
| Scotophilus bat coronavirus 512, complete genome | NC_009657.1 | 41% | 40% | 48% | NA | NA | 40% |
| Bat coronavirus HKU2, complete genome | NC_009988.1 | NA | 48% | 80% | 48% | 48% | 48% |
| Bat coronavirus 1A, complete genome | NC_010437.1 | 41% | 44% | 40% | 48% | 48% | NA |

| | | *In silico* analysis for % identity/homology | | | | | |
|---|---|---|---|---|---|---|---|
| | | Primers-Probe Mix 1 (M1-1) | | | Primers-Probe Mix 2 (M1-2) | | |
| **Microorganism** | **Genbank Accession No.** | **Forward Primer** | **Reverse Primer** | **Probe** | **Forward Primer** | **Reverse Primer** | **Probe** |
| Bat coronavirus HKU8, complete genome | NC_010438.1 | 37% | 40% | NA | NA | NA | 40% |
| Bat coronavirus BM48-31/BGR/2008, complete genome | NC_014470.1 | 59% | 68% | 44% | NA | 57% | NA |
| Rousettus bat coronavirus HKU10, complete genome | NC_018871.1 | NA | 48% | NA | 48% | NA | 40% |
| Bat coronavirus CDPHE15/USA/2006, complete genome | NC_022103.1 | 41% | 44% | NA | NA | 48% | 40% |
| BtMr-AlphaCoV/SAX2011, complete genome | NC_028811.1 | 37% | 40% | 40% | NA | 48% | 48% |
| BtRf-AlphaCoV/HuB2013, complete genome | NC_028814.1 | 44% | 40% | NA | NA | 48% | NA |
| BtRf-AlphaCoV/YN2012, complete genome | NC_028824.1 | NA | 40% | NA | NA | 57% | 48% |
| BtNv-AlphaCoV/SC2013, complete genome | NC_028833.1 | NA | 40% | NA | NA | 48% | 40% |
| Rousettus bat coronavirus isolate GCCD.1 356, complete genome | NC_030886.1 | 37% | 44% | NA | NA | 48% | NA |
| NL63-related bat coronavirus strain BtKYNL63-9a, complete genome | NC_032107.1 | 52% | 48% | 40% | NA | NA | 40% |
| Bat coronavirus isolate PREDICT/PDF-2180, complete genome | NC_034440.1 | NA | 56% | 44% | NA | NA | 40% |

Forward primer, reverse primer, or probe sequences had significant alignments (>80%) with the sequences of several pathogens and these are highlighted in red. Among those are Coronavirus OC43, Coronavirus HKU-1, and *Legionella longbeachae*. These pathogens have been analyzed in more detail in a sequence alignment. No potential unintended cross-reactivity with above listed organisms is expected based on this *in silico* analysis, as they all do not have nearby or correctly oriented primers or probe with significant alignment (>80%) to bi-directionally amplify a PCR product that can be detected on the ViroKey™ SARS-CoV-2 RT-PCR Test. Results confirmed only perfect matches to SARS-CoV-2 and the next closest match is to SARS-CoV-2 ancestor, SARS-coronavirus. *In silico* analysis of pooled microflora showed potential for cross-reactivity, therefore wet testing was performed (described below).

***Analytical specificity (wet testing)***

The ViroKey™ SARS-CoV-2 RT-PCR Test was further evaluated for cross-reactivity with respiratory pathogens commonly present in human respiratory specimens, non-targeted coronaviruses as well as pooled human nasal wash representing the diverse microbial flora in the human respiratory tract. Purified and quantified nucleic acid of the pathogens were added directly into the ViroKey™ SARS-CoV-2 RT-PCR Test

PCR mix in triplicates. All controls performed as expected, and none of the tested pathogens or pooled nasal wash were reactive, (**Table 3**).

**Table 3. Potential cross-reactivity of the ViroKey™ SARS-CoV-2 RT-PCR Test.**

| Tested pathogens, strain (RNA) | | Conc. | *ORF1a* detection (Green channel) | *RdRp* detection (Green channel) | EC detection (Red channel) |
|---|---|---|---|---|---|
| Bacteria | *Haemophilus influenzae* | 1 x $10^5$ copies/µL | 0/3 | 0/3 | 3/3 |
| | *Mycobacterium tuberculosis*, H37Ra | | 0/3 | 0/3 | 3/3 |
| | *Streptococcus pneumoniae* | | 0/3 | 0/3 | 3/3 |
| | *Streptococcus pyogenes* Rosenbach | | 0/3 | 0/3 | 3/3 |
| | *Bordetella pertussis* | | 0/3 | 0/3 | 3/3 |
| | *Pneumocystis jirovecii* | | 0/3 | 0/3 | 3/3 |
| | *Mycoplasma pneumoniae* | | 0/3 | 0/3 | 3/3 |
| | *Legionella pneumophila* | | 0/3 | 0/3 | 3/3 |
| | *Chlamydophila pneumonia* strain CM-1 | | 0/3 | 0/3 | 3/3 |
| Virus | Human coronavirus 229E | | 0/3 | 0/3 | 3/3 |
| | Human coronavirus OC43 | | 0/3 | 0/3 | 3/3 |
| | Human coronavirus HKU1 | | 0/3 | 0/3 | 3/3 |
| | Human parainfluenza virus 2, Greer | | 0/3 | 0/3 | 3/3 |
| | Human parainfluenza virus 3, C243 | | 0/3 | 0/3 | 3/3 |
| | Human parainfluenza virus 4a | | 0/3 | 0/3 | 3/3 |
| | Human parainfluenza virus 4b, CH 19503 | | 0/3 | 0/3 | 3/3 |
| | Influenza A virus (H3N2), A/Aichi/2/68 | | 0/3 | 0/3 | 3/3 |
| | Influenza A virus (H1N1), A/Swine/Iowa/15/30 | | 0/3 | 0/3 | 3/3 |
| | Influenza B virus, B/Lee/40 | | 0/3 | 0/3 | 3/3 |
| | Enterovirus, H | | 0/3 | 0/3 | 3/3 |
| | Human Respiratory syncytial virus, 18537 | | 0/3 | 0/3 | 3/3 |
| | Rhinovirus 57, Ch47 | | 0/3 | 0/3 | 3/3 |
| | Human adenovirus 1, Adenoid 71 | | 0/3 | 0/3 | 3/3 |
| | Human metapneumovirus (hMPV) | | 0/3 | 0/3 | 3/3 |
| Pooled human nasal wash representing the diverse microbial flora in the human respiratory tract | | N/A | 0/3 | 0/3 | 3/3 |

### *Interfering substances*

The potential interfering substances tested at specific concentrations as stated in **Table 4** were found to have not lead to erroneous results with ViroKey™ SARS-CoV-2 RT-PCR Test. The objective of the study was to verify the effect of potentially interfering substances on the performance of ViroKeyTM SARS-CoV-2 RT-PCR Test. Base pool of SARS-CoV-2 positive samples were prepared with SARS-CoV-2 genomic RNA (from American Type Culture Collection (ATCC®) part number VR-1986) spiked into negative nasopharyngeal specimens. For each of the interference substances, 10x stock

concentrations were added to the SARS-CoV-2 positive samples. The test pool contained each interference substance at concentration specified in **Table 4**.

**Table 4. List of tested substances**

| Substance | Active Ingredient/s | Conc. | Detection % (M1-1) | Detection % (M1-2) |
|---|---|---|---|---|
| Nasal Wash (Flo®) | Sodium chloride, potassium chloride, calcium lactate pentahydrate | 15% (v/v) | 100% (3/3) | 100% (3/3) |
| Nasal Spray/drops (Nazolin®) | Oxymetazoline HCl | 15% (v/v) | 100% (3/3) | 100% (3/3) |
| Nasal corticosteroids | Fluticasone | 5% (v/v) | 100% (3/3) | 100% (3/3) |
| Systemic antibacterial | Tobramycin | 4 µg/mL | 100% (3/3) | 100% (3/3) |
| Antiviral drugs | Oseltamivir | 3.3 mg/mL | 100% (3/3) | 100% (3/3) |
| Homeopathic relief (Prospan®) | Extract from ivy leaf (Hedera helix L. leaf), Potassium sorbate, anhydrous citric acid, xanthan gum, cherry flavour, crystallizing sorbitol syrup | 10% (v/v)# | 33% (1/3) | 67% (2/3) |
| | | 5% (v/v) | 100% (3/3) | 100% (3/3) |
| Antimicrobial/antiviral/ anesthetic lozenges (Dorithricin®) | Benzalkonium, Benzocaine, Tyrothricin | 15% (w/v) | 100% (3/3) | 100% (3/3) |
| Whole blood | N.A. | 2% (v/v) | 100% (3/3) | 100% (3/3) |
| Mucin | | 60 µg/mL | 100% (3/3) | 100% (3/3) |

#Prospan® cough syrup interfered with the detection in M1-1 channel when tested at 10% (v/v) but not 5% (v/v).
FLUMIST nasal spray flu vaccine was not tested for its potential interference with ViroKey™ SARS-CoV-2 RT-PCR Test.

### FDA SARS-CoV-2 reference panel testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to corroborate the LoD. The extraction method and instrument used were *Sentosa*® SX Virus Total Nucleic Acid Kit v2.0 and *Sentosa*® SA201 Real-Time PCR Instrument. The results are summarized in **Table 5**.

**Table 5: Summary of LoD confirmation result using the FDA SARS-CoV-2 reference panel**

| Reference Materials Provided by FDA | Specimen Type | Product LoD | Cross-Reactivity |
|---|---|---|---|
| SARS-CoV-2 | Nasopharyngeal Swab | $1.8x10^4$ NDU/mL | N/A |
| MERS-CoV | | N/A | ND |

NDU/mL: RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

## Clinical performance

The clinical validation study of ViroKey™ SARS-CoV-2 RT-PCR Test was conducted on one sample matrix (nasopharyngeal swabs). For ViroKey™ SARS-CoV-2 RT-PCR Test workflow validation, all samples were extracted with *Sentosa*® SX Virus Total Nucleic Acid Kit v2.0 (4x24) manually and detected with ViroKey™ SARS-CoV-2 RT-PCR Test on ABI 7500 Fast Dx.

A total of 118 nasopharyngeal samples were tested and the results are summarized in **Table 6**. The performance of the ViroKey™ SARS- CoV-2 RT-PCR Test were calculated based on the result interpretation table on page 42, where a sample was considered positive if positive signals are detected by M1-1 and/or M1-2 target(s), while a sample was considered negative if no signals are detected by the M1-1 and M1-2 targets. The results from ViroKey™ SARS-CoV-2 RT-PCR Test were compared to an EUA authorized test.

**Table 6. Summary of clinical performance results**

| | | Comparator Results | |
|---|---|---|---|
| | | **Positive** | **Negative** |
| **ViroKey™ SARS-CoV-2 RT-PCR Test** | **Positive** | 35 | 4 |
| | **Negative** | 1 | 78 |

**Sensitivity:** 97.2% (35/36) **95% CI:** 85.8 - 99.5%
**Specificity:** 95.1% (78/82) **95% CI:** 88.1 - 98.1%

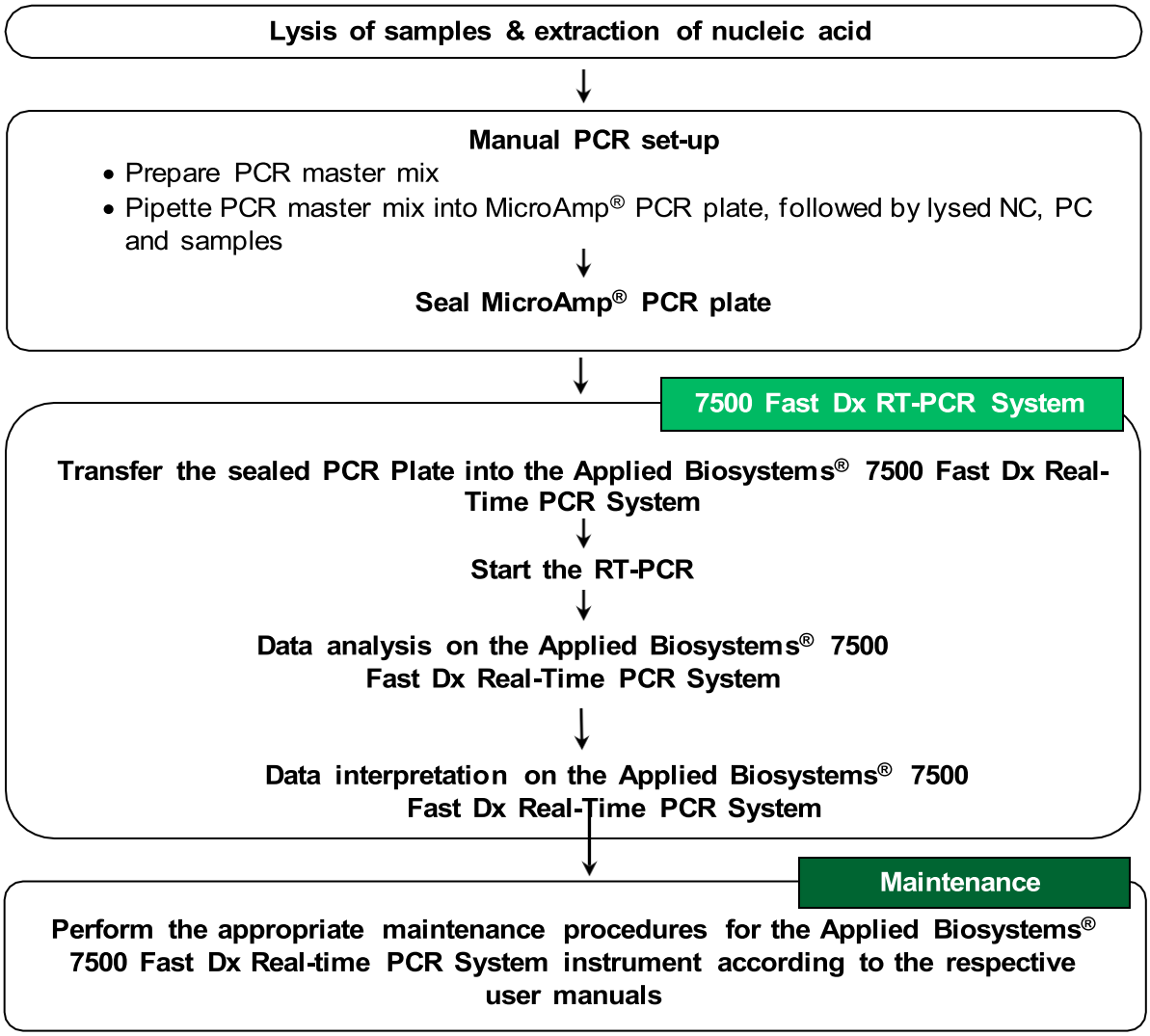
## Workflow

The ViroKey™ SARS-CoV-2 RT-PCR Test manual workflow starts with lysis, followed by the extraction of nucleic acids. After extraction, the user sets up the Reverse Transcription PCR with the extracted nucleic acids in the MicroAmp® Fast Optical 96-Well Reaction Plate. Subsequently the MicroAmp® Fast Optical 96-Well Reaction is sealed and transferred to the Applied Biosystems® 7500 Fast Dx Real-Time PCR System or *Sentosa*® SA201 for PCR amplification followed by data analysis.

The *Sentosa*® SA201 is a rebranded version of the Applied Biosystems® 7500 Fast Dx Real-Time PCR System, thus the workflow is interchangeable between the two instruments. For brevity, only the Applied Biosystems® 7500 Fast Dx Real-Time PCR System workflow will be mentioned in this instructions for use.

An overview of the workflow is provided (see **Flowchart 1)**.

## Flowchart 1: Workflow with ABI 7500 Fast Dx RT-PCT System overview



# Items to be supplied by user

**Table 7. List of items to be supplied by user.**

| Equipment | Description / use | Vela item no. |
|---|---|---|
| Pipettes (adjustable)[i] | For pipetting buffers, reagents and / or samples | N/A |
| Vortex mixer | To mix reagents | N/A |
| Bench top centrifuge[i] | To spin down reagents and remove any bubbles | N/A |
| Applied Biosystems® 7500 Fast Dx Real-Time PCR System[i] | Real-time and end-point thermal cycling using PCR, detection and analysis | N/A |
| *Sentosa*® SA201 Real-Time PCR Instrument | | 400125 |
| Thermomixer[i] | To heat and mix samples | N/A |
| **Accessories / consumables / reagents** | **Description / use** | **Vela item no.** |
| *Sentosa*® SX Virus Total Nucleic Acid Kit v2.0 (4x24) | 4x24 tests | 300353 |
| Nuclease-free water | For RT-PCR set up | N/A |
| Sterile pipette tips with filters | For pipetting buffers, reagents and / or samples | N/A |
| Magnetic stand[ii] | For magnetic beads separation | N/A |
| 1.5 mL DNA low binding tubes[iii] | For storage of extracted nucleic acids and RT-PCR master mixes | N/A |
| Safe-Lock Tubes (1000) | 1000 pieces | 400031 |
| MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL[iv] | N/A | N/A |
| MicroAmp® Optical Adhesive Film[v] | N/A | N/A |
| MicroAmp™ Adhesive Film Applicator[vi] | For applying the MicroAmp® Optical Adhesive Film to seal the MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode | N/A |
| Swabs | For collecting nasopharyngeal samples | N/A |

[i] Ensure that the instruments have been checked and calibrated according to the manufacturer's recommendations.
[ii] DynaMag™-2 Magnet (Cat. No. 12321D) is recommended.
[iii] DNA LoBind Tubes from Eppendorf (Cat. No. 022431021) is validated and recommended.
[iv] MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL from Applied Biosystems® (Cat. No. 4346906) **MUST** be used. Ensure that the correct plates are used.
[v] MicroAmp® Optical Adhesive Film from Applied Biosystems® (Cat. No. 4311971) **MUST** be used. Use only unexpired films.
vi MicroAmp™ Adhesive Film Applicator from Thermo Fisher Scientific (Cat. No. 4333183) is recommended.

# Important notes

## General precautions

- Use sterile pipette tips with filters.
- During manual steps, ensure that the tubes are closed when possible to avoid contamination.
- **Do not mix components from kits with different lot numbers.**
- Proceed continuously from one part of the workflow to the next.

## Specimen collection, handling and storage

- Specimen collection:
  - Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
  - Follow specimen collection device manufacturer instructions for proper collection methods.
- Transporting specimens:
  - Specimens must be packaged, shipped and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens. Store specimens at 2-8°C and ship overnight on ice pack. If a specimen is frozen at ≤-70°C, ship overnight on dry ice.
- Storing specimens:
  - After collection and during transport, the specimen should be stored at 2 − 8°C and all laboratory testing must occur within 72 hours of collection. Refrigerated specimens received outside of this 72-hour window should be rejected.
  - If a delay in shipping is expected, store specimens at ≤-70°C.
  - Specimens received frozen should be stored at ≤-70°C until processing.
  - Store any residual specimens at ≤-70°C.

**NOTE:** Inadequate specimen collection and / or inappropriate specimen processing, storage and transport may yield false negative results.

## Storage of purified nucleic acid

- Purified nucleic acids should be stored at ≤-70°C.

# Protocol: Manual nucleic acid isolation and detection on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System

The *Sentosa*® SX Virus Total Nucleic Acid Kit v2.0 (4x24) is intended for virus total nucleic acid extraction from nasopharyngeal and oropharyngeal swabs for use with the ViroKey™ SARS-CoV-2 RT-PCR Test.

## Important points before starting

- User must be familiar with operating the Applied Biosystems® 7500 Fast Dx Real-Time PCR System or the *Sentosa*® SA201. Please refer to the respective user manuals supplied with the instrument for operating instructions.
- Before beginning the procedure, read the "Important notes" section, page 22.
- Ensure that all reagents of *Sentosa*® SX Virus Total Nucleic Acid Kit v2.0 (4x24) are not precipitated before use.
  - The Virus A1 tubes containing Proteinase K solution require gentle inversion followed by brief centrifugation. The Virus A2 tubes containing magnetic beads require thorough vortexing before use to ensure proper re-suspension.
  - Prior to use, Virus A3 (lyophilized carrier RNA) must be reconstituted with Virus A4 (carrier RNA buffer). Refer to detailed procedure for more information.
  - Mix the buffers in the bottles by gentle swirling, ensuring no foam or bubbles are present.
- Each tube of reagent in the ViroKey™ SARS-CoV-2 RT-PCR Test is designed for 50 reactions.
- RNA4 M3 is an enzyme, which is in liquid state. Except RNA4 M3, the rest of the ViroKey™ SARS-CoV-2 RT-PCR Test components should be thawed at room temperature (approximately 15°C – 25°C) for up to 30 minutes.
- RNA4 M3 should be used directly out of the freezer or kept on ice when performing reagent preparation. Handle carefully to avoid contamination and store the remaining RNA4 M3 immediately after use at ≤ −20°C for subsequent reactions.
- All reagents should not be subjected to more than five (5) freeze-thaw cycles as this may compromise assay performance.
- For software, use current version or higher

  SDS Software Version 1.4.1
  *Sentosa*® SA201 Series Software Version 1.0.1

## Procedure

### *1. Sample pre-treatment and lysis*

1.1. Prepare the reagents from the *Sentosa*® SX Virus Total Nucleic Acid Kit v2.0 (4x24) according to step 1.2.

1.2. Reconstitute Virus A3 (lyophilized carrier RNA) with Virus A4 (carrier RNA buffer) as described below.

- Briefly spin down Virus A3 and Virus A4 for 5 seconds.
- Add 125 µL of Virus A4 (24) to each tube of Virus A3 (24).
- The reconstituted Virus A3 should be mixed by pulse vortexing for 30 seconds followed by brief centrifugation.

1.3. Prepare sufficient lysis master mix according to the table below. Scale according to the number of samples that you need to process.

| Reagents | 1 sample | 24 samples | 48 samples |
|---|---|---|---|
| Virus B1 (24) | 211 µL | 5.275 mL | 10.55 mL |
| Virus A1 (24) | 10 µL | 250 µL | 0.5 mL |
| Reconstituted Virus A3 (24) | 4 µL | 100 µL | 200 µL |
| EC8 | 7 µL | 175 µL | 350 µL |

**NOTE:**

- Virus A1 tube containing Proteinase K solution should be mixed by gentle inversion followed by brief centrifugation.
- Ensure that the lysis master mix is added to the samples within 30 minutes of preparation, as Virus A1 may self-digest when incubating in Virus B1 (lysis buffer) without the sample.

1.4. Transfer 230 µL of lysis master mix and 230 µL of samples into fresh 1.5 mL safe-lock microtubes.

1.5. Pulse vortex the 1.5 mL safe-lock microtubes for 10 seconds followed by brief centrifugation to collect the contents at the bottom. Incubate the tubes at 56°C for 10 minutes under 1,200 rpm agitation using a thermomixer.

1.6. Centrifuge the samples briefly to collect the contents at the bottom of the tubes.

1.7. Proceed to **section 2**, “Nucleic acid extraction”.

## 2. *Nucleic acid extraction*

2.1. Prepare sufficient binding buffer mix according to the table below. Scale according to the number of samples that will be processed.

| Reagents | 1 sample | 24 samples | 48 samples |
|---|---|---|---|
| Virus A2 (24) | 19.5 µL | 570 µL | 1.14 mL |
| Virus B2 (24) | 580.5 µL | 17 mL | 34 mL |

**NOTE:**
- Virus A2 tube containing magnetic beads should be mixed thoroughly before addition to Virus B2.

2.2. Add 600 µL of binding buffer mix to each sample and vortex the tubes for 1 minute.

2.3. Place tubes on a magnetic rack for 2 minutes.

2.4. Carefully discard the solution without removing any magnetic beads.

2.5. Add 600 µL of Virus B3 to each sample and vortex the tubes for 1 minute.

2.6. Place tubes on a magnetic rack for 2 minutes.

2.7. Carefully discard the solution without removing any magnetic beads.

2.8. Add 600 µL of Virus B4 to each sample and vortex the tubes for 1 minute.

2.9. Place tubes on a magnetic rack for 2 minutes.

2.10. Carefully discard the solution without removing any magnetic beads.

2.11. **IMPORTANT:** To remove residual solution, dry samples at 55°C for 3 minutes with mixing at 600 rpm on a thermomixer with lid left open.
**NOTE: DO NOT** over-dry the magnetic beads.

2.12. To elute nucleic acid, add 50 µL of Virus B6 to the magnetic beads of each sample, vortex for 5 seconds and incubate the tubes at 25°C for 5 minutes with 1,000 rpm agitation using a thermomixer.

2.13. Place the tubes on a magnetic rack and transfer all solution to fresh 1.5 mL DNA LoBind tubes, taking care not to remove any magnetic beads.

2.14. Proceed to **section 3**, “RT-PCR set up”.

## *3. RT-PCR set up*

3.1. Prepare PCR reagents from the ViroKey™ SARS-CoV-2 RT-PCR Test. Pulse vortex M1 for 10 seconds, and mix M2 by gentle inversion. Centrifuge M1 and M2 briefly to collect the contents at the bottom of the tubes.

3.2. Prepare the appropriate amounts of PCR master mix in fresh 1.5 DNA LoBind Tubes. Refer to the table below for reaction set-up volume or scale according to the number of samples to be processed. Prepare two master mixes, one for each target (M1-1 and M1-2).

| Components of PCR Master Mix | 1 test | 25 tests | 50 tests |
|---|---|---|---|
| SARS-CoV-2 M1-1 / SARS-CoV-2 M1-2 | 1 µL | 28 µL | 55 µL |
| RNA4 M2 | 12.5 µL | 350 µL | 687.5 µL |
| RNA4 M3 | 2 µL | 56 µL | 110 µL |

3.3. Mix the PCR master mix sufficiently by gentle inversion. Centrifuge the PCR master mix briefly to collect the contents at the bottom of the tube.

3.4. Pipette 15.5 µL of PCR master mix into the wells of the MicroAmp® Fast Optical 96-Well Reaction plate and transfer 10 µL of sample eluate from step 2.13 into each well containing PCR master mix.

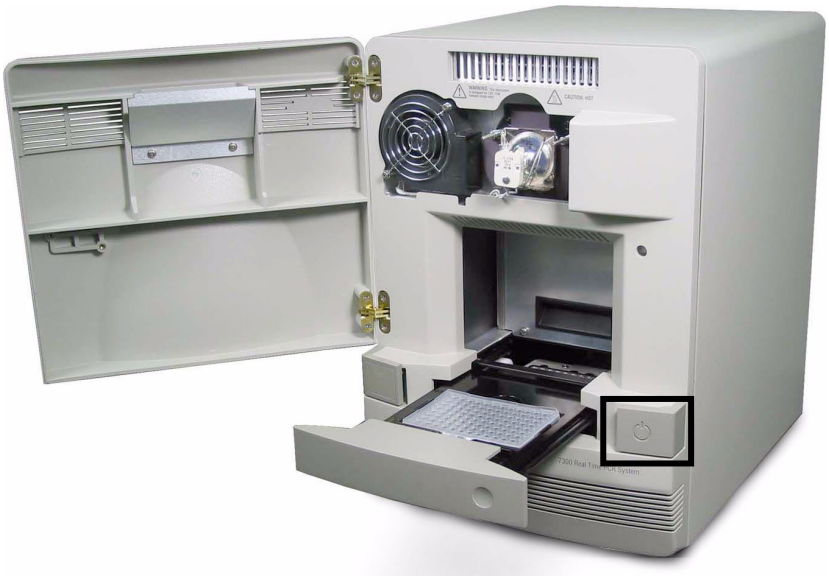
3.5. For PC and NC, pipette of 5 µL of PC and NC from their respective tubes into their respective wells containing the PCR master mix on the PCR plate and add 1 µL of EC from EC tube into each well. Add 4 µL of nuclease-free water to each well.

3.6. Seal the MicroAmp® Fast Optical 96-Well Reaction plate with the MicroAmp® Fast Optical Adhesive Film and place the sealed plate into the Applied Biosystems® 7500 Fast Dx Real-time PCR System.
**NOTE:** Refer to the MicroAmp® Fast Optical Adhesive Film user manual for details on proper sealing. **DO NOT** use expired adhesive films as proper sealing is essential for the RT-PCR.

3.7. Proceed to **section 4**, “PCR on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System”.

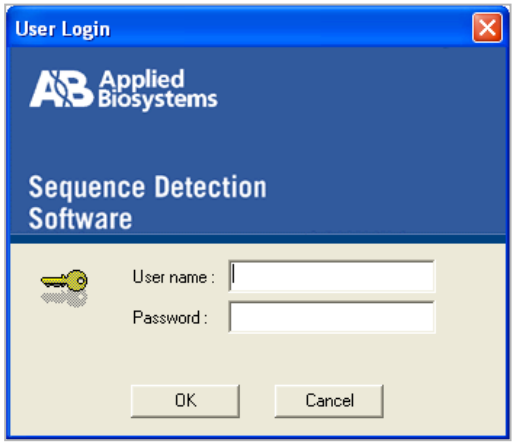
## *4. PCR on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System*

4.1. Turn on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System by pressing the power button on the instrument, and wait for the initiation procedure to be completed.
**NOTE:** Ensure the green indicator is lit and not flashing.

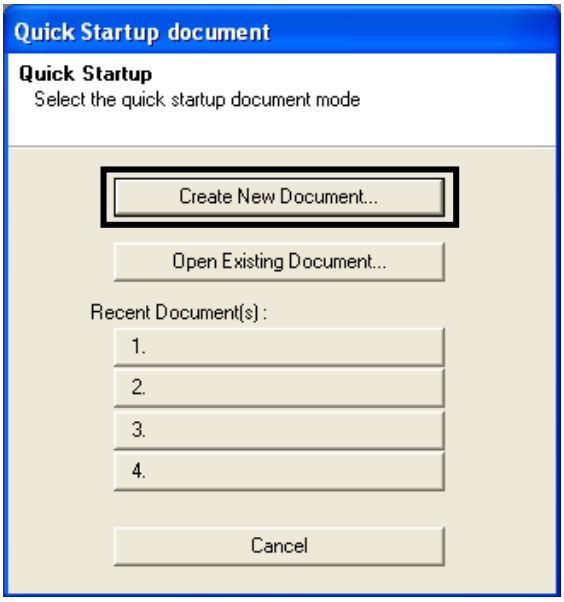


4.2. Run Reverse Transcription PCR

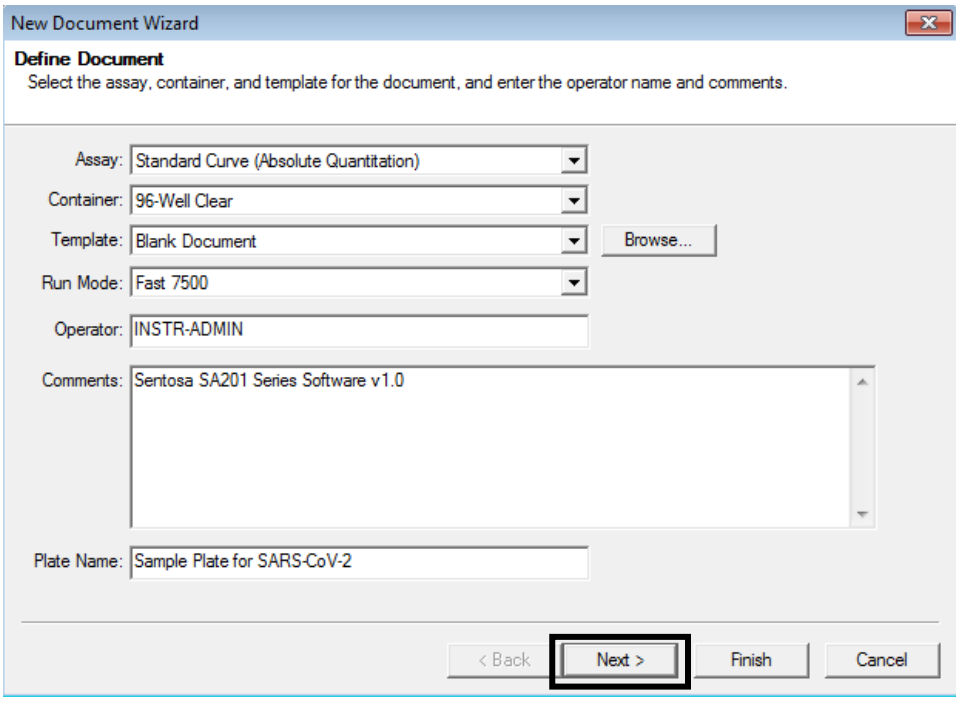
4.2.1. On the instrument's computer, launch the ABI 7500 Fast System SDS Software by double-clicking the  icon. The *"Login"* window opens, type the account name and password, and then click on *"OK"*.



4.2.2. In the "Quick Startup document" dialog box, select "*Create New Document*".



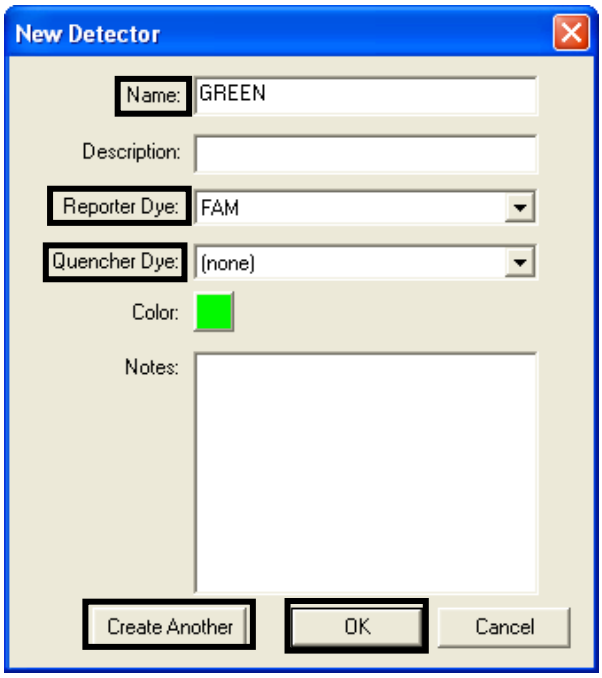

4.2.3. A "*New Document Wizard*" window appears for "*Define Document*". In the "*Assay*" drop-down list, select "*Standard Curve (Absolute Quantitation)*". The default setting for "*Container*" should be "*96-Well Clear*". The default setting for "*Template*" should be "*Blank Document*". In the "*Run Mode*" drop-down list, select "*Standard 7500*". Enter the name of the operator and the plate in the "*Operator*" and "*Plate Name*" fields respectively. Click "*Next*".



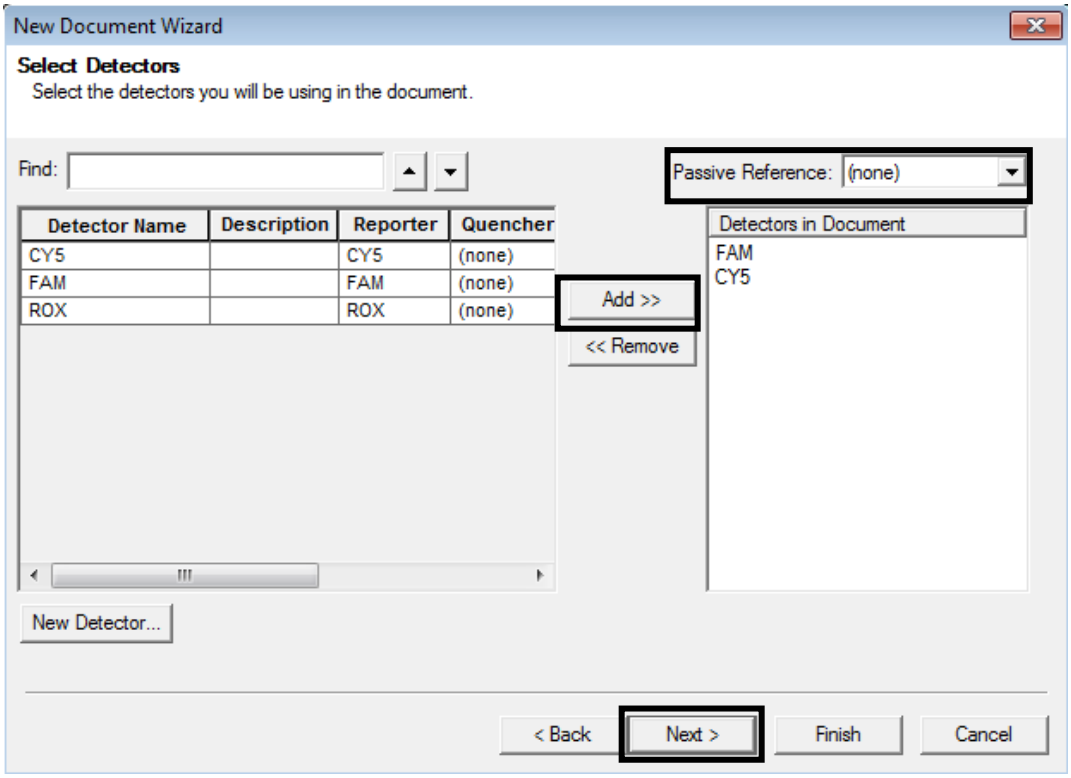
4.2.4. A "*New Document Wizard*" window appears for "*Select Detectors*". Click "*New Detector*" to create new "*GREEN*" and "*RED*" detectors, if needed, according to the table below:

| Detector Name | Reporter Dye | Quencher Dye |
|---|---|---|
| GREEN | FAM | (none) |
| RED | CY5 | (none) |

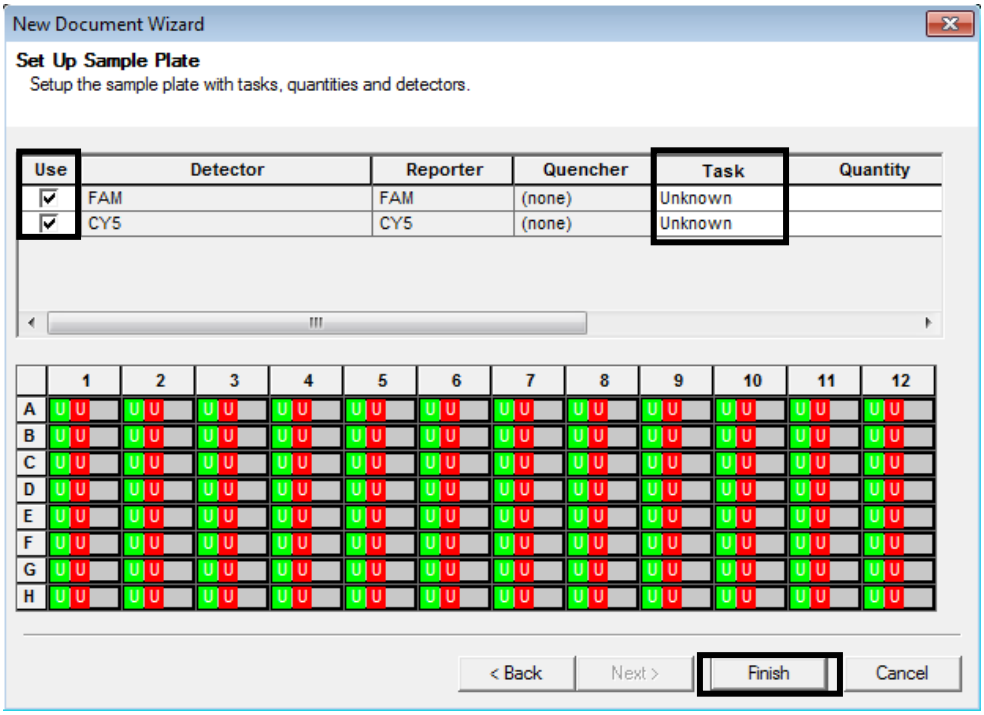
Select the *"Reporter Dye"* from the drop-down list. Select the *"Quencher Dye"* as *"None"*. Select the *"Color"* to match the detector. Click *"Create Another"* to specify new detector *"Name"*, *"Reporter Dye"* and *"Quencher Dye"*. Click *"OK"* after all detectors are created.



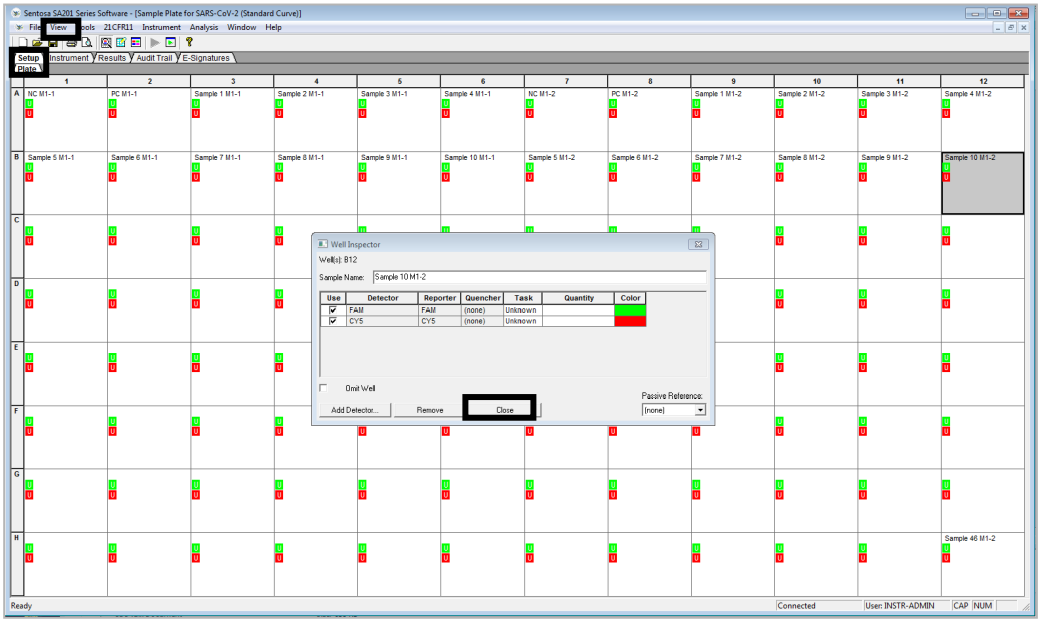
4.2.5. The "*New Document Wizard*" window for "*Select Detectors*" appears. Select the detectors and click "*Add*". In the "*Passive Reference*" drop-down list, select "*(none)*". Click "*Next*".



4.2.6. A "*New Document Wizard*" window for "*Set Up Sample Plate*" appears. Select all wells and check the "*Use*" boxes for the detectors. The default setting for "*Task*" should be "*Unknown*". Click "*Finish*". The SDS Software will create the plate document.



4.2.7. A window for plate document appears. In the window, select "*Setup*" tab, and the "*Plate*" tab should be displayed. Name the wells of the plate by selecting them, then click on "*View*" menu, followed by "*Well Inspector*".

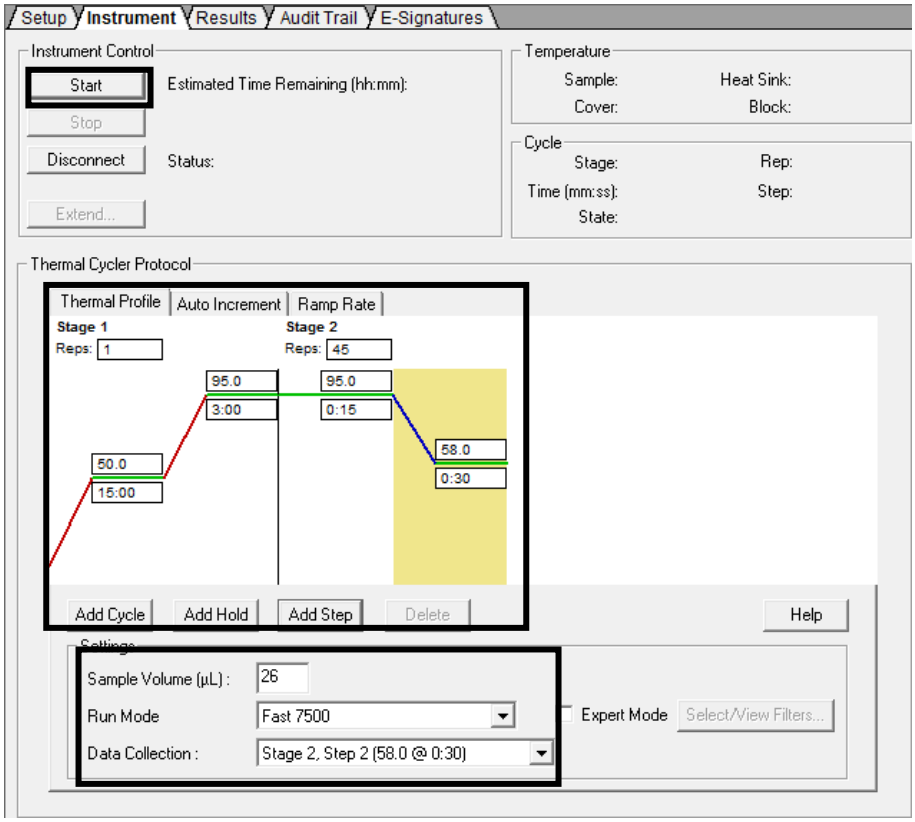



Enter the sample name for each well and verify the information of the well(s). Click *"Close"*.

4.2.8. The thermal cycling conditions need to be manually specified according to the table below.

| Stage | Reps | Temperature (°C) | Duration (min) |
|---|---|---|---|
| 1 | 1 | 50 | 15:00 |
| 2 | 1 | 95 | 3:00 |
| 3 | 45 | 95 | 0:15 |
| | | 58 | 0:30 |

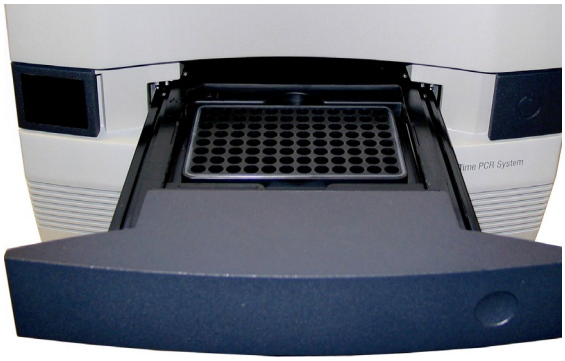
Under the "*Instrument*" tab, enter the thermal cycling condition values in the boxes. Accept the default settings for *"Auto Increment"* and *"Ramp Rate"* for all stages.

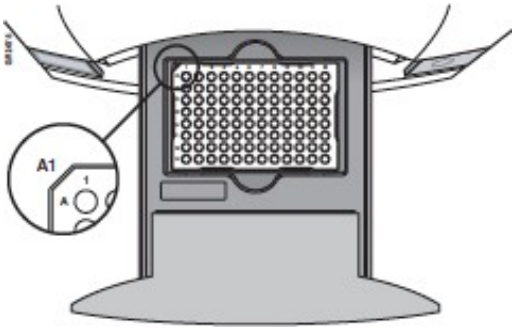



Specify the *"Sample Volume"* as 26 µL. The default setting for *"Run Mode"* should be *"Fast 7500"*. In the *"Data Collection"* drop-down list, select *"Stage 3, Step 2 (58.0 @ 0:30)"*.

4.2.9. Save the run file in *"sds"* format. The plate document can also be saved as a template document for future use. To do this, save the template file in *"sdt"* format in a preferred folder.

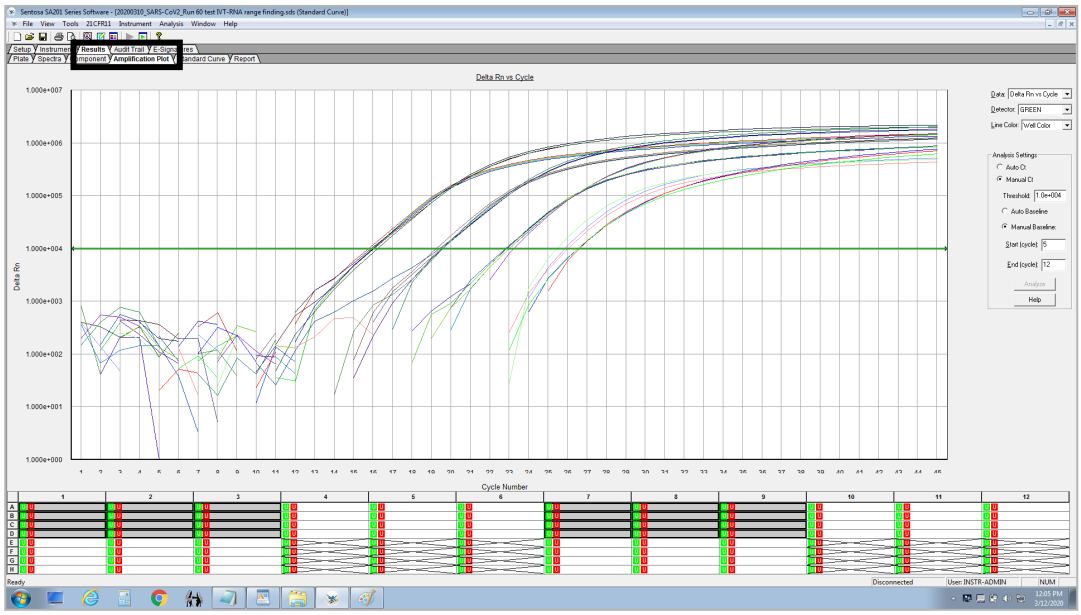
4.2.10. At the front of the Applied Biosystems® 7500 Fast Dx Real-Time PCR System, push to open the plate holder tray and load the plate into the precision plate holder. Ensure that the plate is properly aligned in the holder.



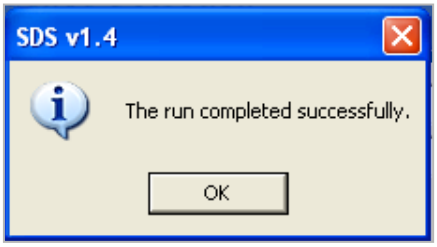



4.2.11. Under *"Instrument Control"* on the *"Instrument"* tab, click *"Start"*.

4.2.12. Information on real-time status of the run based on fluorescence emissions is displayed in the *"Results"* tab, under *"Amplification Plot"*.



4.2.13. At the end of the run, a message appears, indicating whether or not the run is successful. All data generated is saved in the *"sds"* file specified in step 4.2.9.

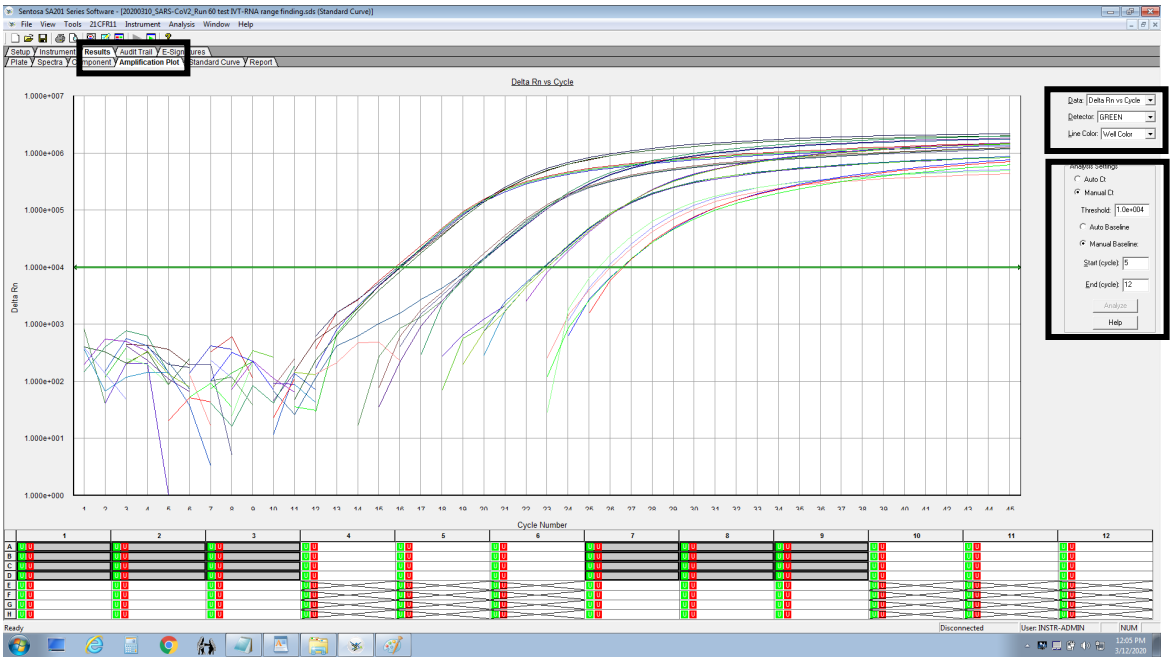



4.2.14. After the run is completed, proceed to **section 5**, "Data analysis on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System".

## 5. *Data analysis on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System*

**NOTE:** Please take note of where the samples were loaded in the MicroAmp® Fast Optical 96-Well Reaction plate and analyze the results accordingly.

5.1. In the run window select the *"Results"* tab, followed by the *"Amplification Plot"* tab. Select *"Delta Rn vs Cycle"* in the *"Data"* drop-down list. In the *"Detector"* drop-down list, select a detector (*"GREEN" or "RED"*).



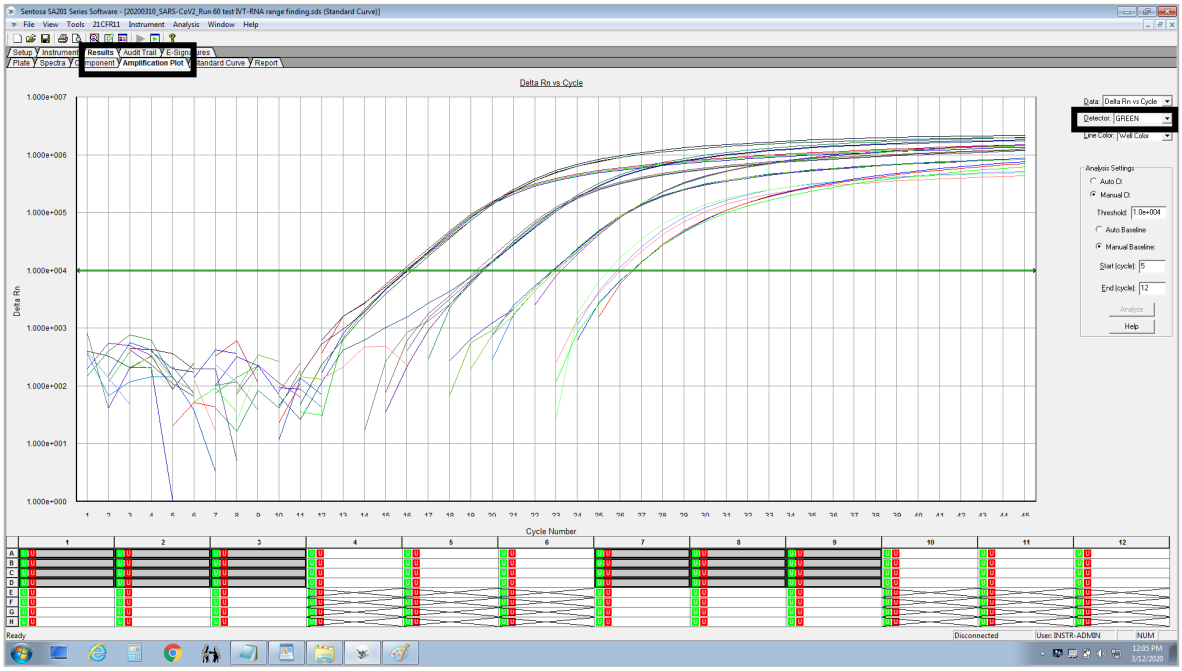

Set the threshold and baseline for the detectors according to the table below:

| Detector | Threshold | Baseline | |
|---|---|---|---|
| | | Start (cycle) | End (cycle) |
| **GREEN M1-1** | 1.00e+004 | 5 | 12 |
| **GREEN M1-2** | 2.00e+004 | 5 | 12 |
| **RED** | 5.00e+003 | 5 | 12 |

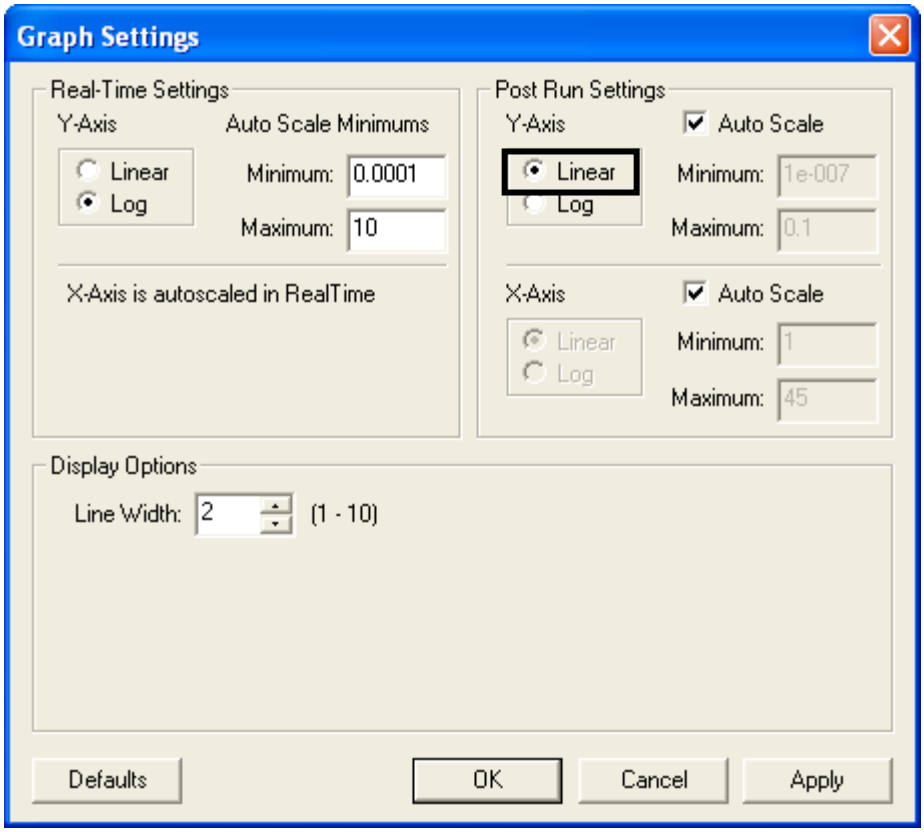
To set the threshold for the detector, select *"Manual Ct"* under *"Analysis Settings"* and enter the value. To set the baseline for the detector, select *"Manual Baseline"* under *"Analysis Settings"* and enter the values. Click *"Analyze"*. After which, the red threshold line turns green.

Repeat these steps to set the threshold and baseline values for all detectors.

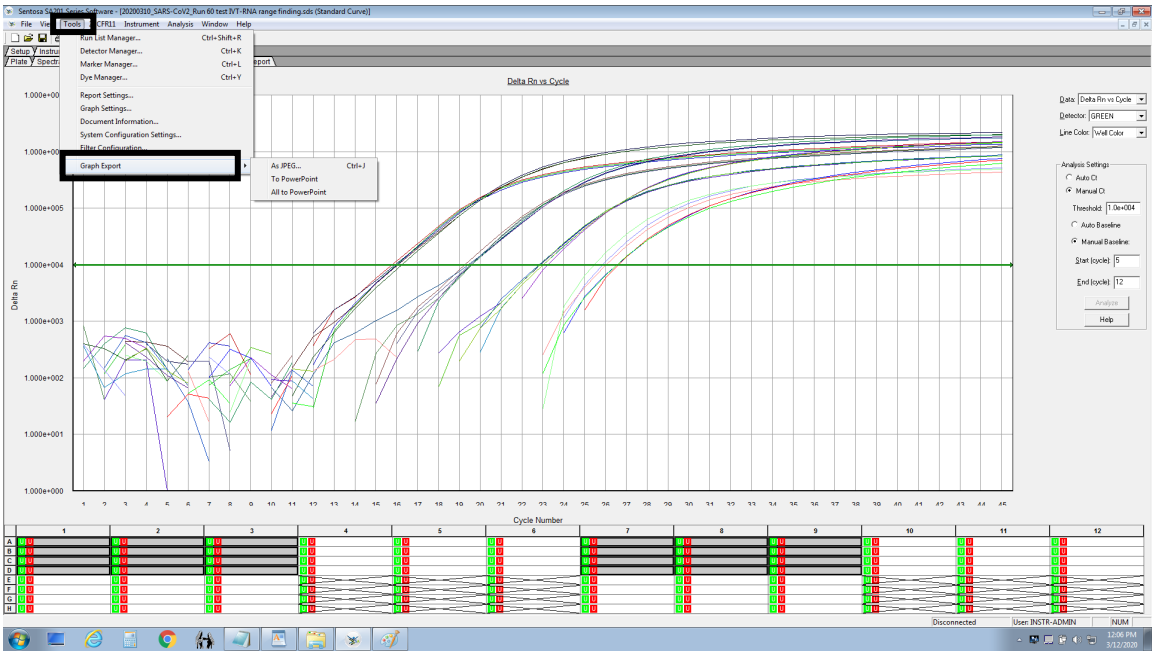
5.2. To generate graphs for specific samples or target channel, select the corresponding sample wells at the bottom of the window, then choose the desired target channel from the *"Detector"* drop-down list. Select *"GREEN"* for the analysis of the SARS-CoV-2 targets (*ORF1a* and *RdRp*) and *"RED"* for the analysis of the extraction control.



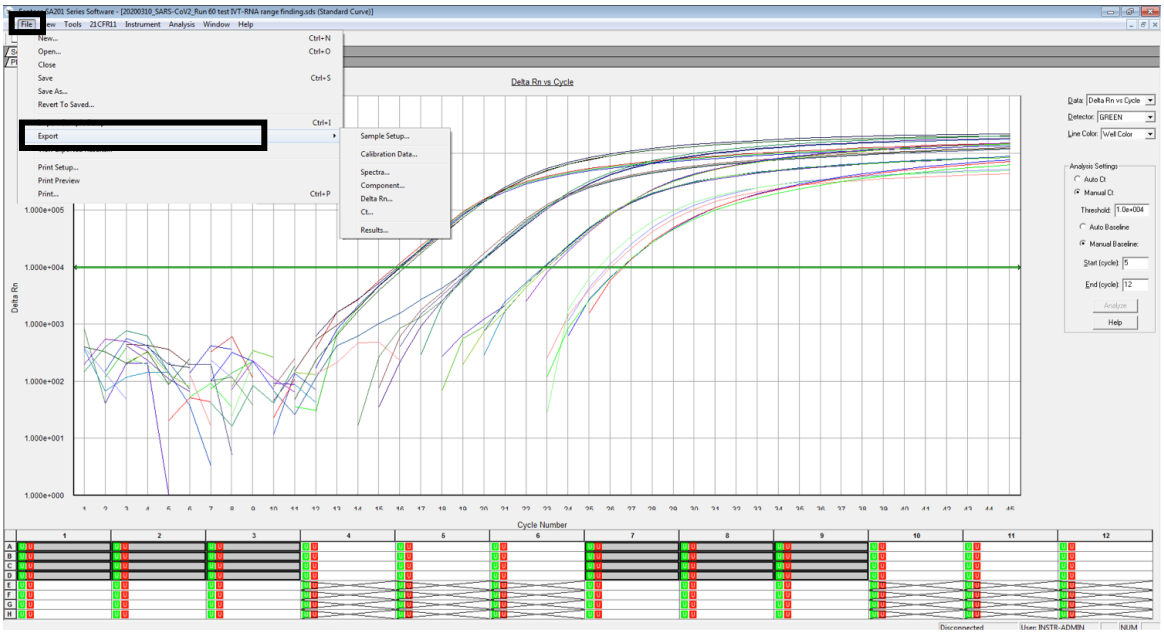
5.3. Right click on the graph to select “Graph setting”. In “Post Run setting” category, choose “Linear” for Y-axis.



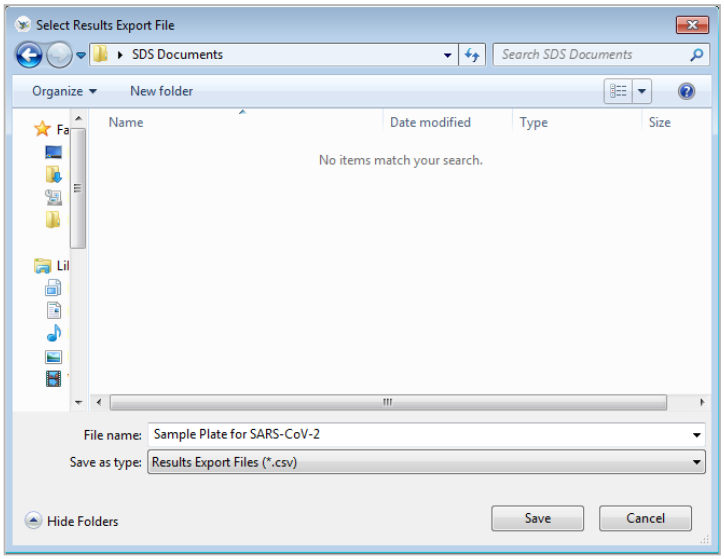
5.4. To export the graphs for the run, select *"Tools"* menu -> *"Graph Export"*, then choose the desired format to export the graph in.




5.5. To export results, save the *".sds"* file first. Select *"File"* menu -> *"Export"*, then choose the desired information to export.



5.6. The *"Select Results Export"* pop-up window appears. Accept the default setting for *"Save as type"*. Navigate to the desired folder and enter the name of the result export file (*".csv"*). Click *"Save"*.




5.7. After the data has been analyzed, proceed to **section 6**, "Data interpretation after analysis on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System".

## 6. *Data interpretation after analysis on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System*

*Negativity / positivity*

The $C_T$ range to define negativity / positivity for negative control, positive control and sample is listed in the table below. If $C_T$ falls within the range, it is defined as positive (+); if $C_T$ falls out of the range or no $C_T$ is obtained, it is defined as negative (-). The green fluorescence channel detects the *ORF1a* and *RdRp* genes while the red fluorescence channel detects the extraction control.

| Fluorescence channel | $C_t$ range for negativity (-) and positivity (+) | | | | | |
|---|---|---|---|---|---|---|
| | Negative control | | Positive control | | Samples | |
| | - | + | - | + | - | + |
| Green M1-2 (SARS-CoV-2 Orfla) | ≥ 40.0 or Undet or no $C_t$ | < 40.0 | < 24.0, > 30.0 or no $C_t$ | 24.0 – 30.0 | > 40.0, < 10.0 or no $C_t$ | 10.0 – 40.0 |
| Green M1-1 (SARS-CoV-2 RdRp) | ≥ 40.0 or no $C_t$ | < 40.0 | < 24.0, > 30.0 or no $C_t$ | 24.0 – 30.0 | > 40.0, < 10.0 or no $C_t$ | 10.0 – 40.0 |
| Red (EC) | < 23.0, > 29.0 or no $C_t$ | 23.0 – 29.0 | < 23.0, > 29.0 or no $C_t$ | 23.0 – 29.0 | < 20.0, > 40.0 or no $C_t$ | 20.0 – 40.0 |

Note: Ct range updated after verification and validation. No $C_t$ is equivalent to Undet: Undetermined.

*Run validity*

Please refer to the table below for run validity interpretation.

| Control | SARS-CoV-2 M1-1 (Green) | SARS-CoV-2 M1-2 (Green) | Red* | Interpretation |
|---|---|---|---|---|
| Negative control | - | - | + | Run valid (proceed to PC) |
| | + | - | +/- | Run invalid. Repeat run. |
| | - | + | +/- | |
| | + | + | +/- | |
| | - | - | - | |
| Positive control | + | + | +/- | Run valid (proceed to positive sample control) |
| | + | - | +/- | Run invalid. Repeat run. |
| | - | + | +/- | |
| | - | - | +/- | |
| SARS-CoV-2 positive sample control | + | + | +/- | Run valid (proceed to result interpretation of samples) |
| | + | - | +/- | Run invalid. Repeat run. |
| | - | + | +/- | |
| | - | - | +/- | |

*Result interpretation of samples*

Please refer to the table below for result analysis. All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted

| SARS-CoV-2 M1-1 (Green) | SARS-CoV-2 M1-2 (Green) | Red* | Interpretation |
|---|---|---|---|
| + | + | M1-1: +/-<br>M1-2: +/- | All Target Results are valid. Result for SARS-CoV-2 RNA is Detected. |
| - | + | M1-1: +<br>M1-2: +/- | All Target Results are valid.<br>Result for SARS-CoV-2 RNA is Detected. |
| + | - | M1-1: +/-<br>M1-2: + | All Target Results are valid.<br>Result for SARS-CoV-2 RNA is Detected. |
| - | - | M1-1: +<br>M1-2: + | All Target Results are valid.<br>Result for SARS-CoV-2 RNA is Not Detected. |
| - | + | M1-1: -<br>M1-2: +/- | M1-1 Target Result is invalid.<br>Result for SARS-CoV-2 RNA is Detected. |
| + | - | M1-1: +/-<br>M1-2: - | M1-2 Target Result is invalid.<br>Result for SARS-CoV-2 RNA is Detected. |
| - | - | M1-1: +<br>M1-2: - | M1-2 Target Result is invalid.<br>Result inconclusive. Sample should be retested. If the result is still invalid, a new specimen should be obtained. |
| - | - | M1-1: -<br>M1-2: + | M1-1 Target Result is invalid.<br>Result inconclusive. Sample should be retested. If the result is still invalid, a new specimen should be obtained. |
| - | - | M1-1: -<br>M1-2: - | All Target Results are invalid.<br>Sample should be retested. If the result is still invalid, a new specimen should be obtained. |

*For positive samples, the fluorescence channel Cycling Red may be negative due to competition with the target channels.

**Run**: Whole run on the MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode

**Test**: Test to which the NC / PC belongs

**Sample**: Single sample in one well of the MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode

## Instrument maintenance

After every run, discard used sample tubes, plates, reagents and tips according to the local safety regulations. All samples and waste should be considered potentially infectious.

Dispose the liquid waste according to the local safety and environment regulations.

Perform regular cleaning of the Applied Biosystems® 7500 Fast Dx Real-Time PCR System instrument after each run. Refer to the respective instrument user manual for detailed procedures.

Ensure that maintenance is performed regularly to minimize the risk of error.

Always wear the appropriate personal protective equipment (PPE: lab coat, gloves, goggles, respirator mask, surgical mask) during cleaning / maintenance procedures.

# Troubleshooting guide

The troubleshooting guide may be helpful in solving any problems that may arise. For more information, please contact the authorized Vela Diagnostics representative. Vela Diagnostics Service and Support is always ready to answer any questions about the information and protocols in this instructions for use or sample and assay technologies (for contact information, refer to the back cover).

| | | Comments and recommended actions |
|---|---|---|
| **1.** | **General handling** | |
| a) | Error message displayed on the screen | When an error message is displayed during a protocol run, please refer to the instrument user manuals. |
| **2.** | **Precipitates in the reagents of the *Sentosa*® SX Virus (4x24) Acid Kit v2.0** | |
| a) | Storage of reagents | Reagents might precipitate upon storage. If required, incubate the reagents in a water bath at 37°C for 30 minutes and shake occasionally to dissolve the precipitates. |
| **3.** | **Consistent high $C_T$ values observed for samples** | |
| a) | Magnetic beads were not completely re-suspended | Virus A2 (magnetic beads) requires thorough vortexing before use to ensure proper re-suspension. |
| b) | Frozen samples were not mixed properly after thawing | Thaw frozen samples with mild agitation to ensure thorough mixing. |
| c) | Degraded nucleic acids | Ensure that samples are stored correctly and not subjected to multiple freeze-thaw cycles. Repeat the extraction procedure with new samples. |
| d) | Incomplete sample lysis | Ensure that Virus B1 (lysis buffer) does not contain precipitates. If required, incubate it in a water bath at 37°C for 30 minutes and shake occasionally to dissolve the precipitates. |
| **4.** | **No signal with positive control (PC) in the green or red fluorescence channels** | |
| a) | PCR conditions do not comply with the protocol | Ensure that the correct thermal cycling conditions are input into the SDS Software. Please refer to **section 4** “PCR on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System” on page 27. |

| | | |
|---|---|---|
| b) | Incorrect PCR configuration | Ensure that the correct thermal cycling conditions are input into the SDS Software. Please refer to **section 4**. "PCR on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System" on page 27. |
| c) | Storage conditions for one or more components did not comply with the instructions given in the "Storage" section | Check the storage condition (refer to the kit label) of the reagents and use a new kit, if necessary. |
| d) | Extraction / assay kit has expired | Check the expiration date (refer to the kit label) of the reagents and use a new kit, if necessary. |

**5. Weak or no signal of the extraction control (EC) in the Red fluorescence channel subjected to extraction using the *Sentosa*® SX Virus Total Nucleic Acid Kit v2.0 (4x24)**

| | | |
|---|---|---|
| a) | PCR conditions do not comply with the protocol | Ensure that the correct thermal cycling conditions are input into the SDS Software. Please refer to **section 4.** "PCR on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System" on page 27.<br><br>For PCR configuration, please refer to section 4 of the troubleshooting guide. |
| b) | PCR inhibition | Dilute the extraction eluent 10 times and repeat the PCR.<br><br>Collect new sample and repeat the *Sentosa*® workflow. |
| c) | EC is not added into the sample | Ensure that EC was added during the sample pre-treatment and lysis steps. |
| d) | Loss of nucleic acid during extraction | No signal from the extraction control may indicate the loss of nucleic acid during the extraction. Follow the instructions for nucleic acid extraction in **section 2** on page 25 closely.<br><br>Refer to section 3 of the troubleshooting guide. |
| e) | Storage conditions for one or more kit components did not comply with the instructions given in the "Storage" section | Check the storage condition (refer to the kit label) of the reagents and use a new kit if necessary. |

| | | |
|---|---|---|
| f) | Extraction / assay kit has expired | Check the expiration date (refer to the kit label) of the reagents and use a new kit if necessary. |
| **6.** | **Signals with the negative control in the Green or Red fluorescence channels of the analytical PCR** | |
| a) | Contamination occurred during extraction / PCR set-up | Repeat the extraction and PCR protocols with new reagents.<br>Ensure that the workspace and instruments are decontaminated as recommended. |

## References

1) Pyrc, K., Berkhout B. and van der Hoek L. (2006). The Novel Human Coronaviruses NL63 and HKU1. *J Virol*. 81(7), 3051-3057.
2) Trombetta, H., Faggion, H.Z., Leotte, J., Nogueira, M.B., Vidal, L.R.R. and Raboni, S.M. (2016). Human coronavirus and severe acute respiratory infection in Southern Brazil. *Pathog Glob Health* 110(3): 113-118.
3) Xia, S., Yan, L., Xu, W., Agrawal, A.S., Algaissi, A., Tseng, C.K., Wang, Q., Du, L., Tan, W., Wilson, I.A., Jiang, S., Yang, B. and Lu, L. (2019). A pan-coronavirus fusion inhibitor targeting the HR1 domain of human coronavirus spike. *Sci Adv* 5(4): eaav4580.
4) Wang, W., Tang J. and Wei, F. (2020). Updated understanding of the outbreak of 2019 novel coronavirus (2019-nCoV) in Wuhan, China. *J Med Virol*. https://doi.org/10.1002/jmv.25689.
5) Gorbalenya, A.E., Baker, S.C., Baric R.S., de Groot, R.J., Drosten, C., Gulyaeva, A.A., Haagmans, B.L., Lauber, C., Leontovich, A.M., Neuman, B.W., Penzar, D., Perlman, S., Poon, L.L.M., Samborskiy, D., Sidorov, I.A., Sola, I. and Ziebuhr, J. (2020). Severe acute respiratory syndrome related coronavirus: The species and its viruses – a statement of the Coronavirus Study Group. *bioRxiv.* https://doi.org/10.1101/2020.02.07.937862.

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2. Vela Diagnostics grants no license under any of its intellectual property to use or incorporate the enclosed components of this test with any components not included within this test except as described in the ViroKey™ SARS-CoV-2 RT-PCR Test instructions for use and additional protocols.
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6. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above.
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For updated license terms, please contact the authorized Vela Diagnostics representative.



2021-06-22

# Contacts

**Manufacturer**
Vela Operations Singapore Pte Ltd #05-07 The Kendall
50 Science Park Road Singapore 117406
Tel: +65 6672 6060
Fax: +65 6672 6066
General inquiry email: infoAPAC@veladx.com

**Distributor**
Vela Diagnostics USA, Inc. 353C Route 46 West
Suite 250 Fairfield, NJ 07004
Orders: +1 877 593 7528
Technical: +1 877 593 7528
Fax: +1 973 521 7077
General inquiry email: infoUSA@veladx.com

**Regional technical support**
United States of America: support.us@veladx.com

**Visit our website:** www.veladx.com

**For use under an Emergency Use Authorization Only**

**Rx Only**

**For Prescription Use Only.**

**IVD**

For *in vitro* diagnostic (IVD) use

Consult instructions for use. Electronic Instruction for Use (IFU) is available on Veladx.com at https://www.veladx.com/product/qpcr-respiratory-viruses/virokey-sars-cov-2-rt-pcr-test.html

## Intended use

ViroKey™ SARS-CoV-2 RT-PCR Test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, oropharyngeal swab specimens and nasopharyngeal wash/aspirate or nasal aspirate specimens) collected from individuals suspected of COIV-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C.§263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The ViroKey™ SARS-CoV-2 RT-PCR Test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The ViroKey™ SARS-CoV-2 RT-PCR Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

## Principle

ViroKey™ SARS-CoV-2 RT-PCR Test uses TaqMan® probe chemistry for real-time RT-PCR detection of viral nucleic acid extracted from upper respiratory specimens using the *Sentosa*® SX Virus Total Nucleic Acid Kit (4x24) v2.0. The product includes two sets of oligonucleotide primer and probe mixtures (PPM) for the detection of either *RdRp* (tube M1-1) or *ORF1a* (tube M1-2) sequences from SARS-CoV-2 viral RNA with FAM reporter dye in the Green fluorescence channel. An extraction control (EC) consisting of a non-human synthetic DNA fragment is multiplex with each PPM using another reporter dye in the Red fluorescence channel to consolidate the reaction into a single well.

Additional materials in the ViroKey™ SARS-CoV-2 RT-PCR Test include enzyme mixes (tubes M2 and M3), Negative Control (tube NC), Positive control (tube PC) and extraction control (tube EC) to be added to each sample.

## Materials Provided and Storage

*Table 1. ViroKey™ SARS-CoV-2 RT-PCR Test (PN: 300681)*

| Kit item no. | Item | Quantity | Volume / tube | Shipping Condition | Storage Condition |
|---|---|---|---|---|---|
| 300681 | SARS-CoV-2 M1-1 | 4 | 60 µL | Dry ice | -25°C – -15°C |
| | SARS-CoV-2 M1-2 | 4 | 60 µL | Dry ice | -25°C – -15°C |
| | RNA4 M2 | 8 | 700 µL | Dry ice | -25°C – -15°C |
| | RNA4 M3 | 4 | 240 µL | Dry ice | -25°C – -15°C |
| | NC5 | 4 | 600 µL | Dry ice | -25°C – -15°C |
| | SARS-CoV-2 PC | 4 | 600 µL | Dry ice | -25°C – -15°C |
| | EC8 | 4 | 600 µL | Dry ice | -25°C – -15°C |

## Warnings and Precautions

- This test is for use under an Emergency Use Authorization.
- For Prescription Use Only.
- For *in vitro* diagnostic use only (IVD)
- The ViroKey™ SARS-CoV-2 RT-PCR Test has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. 263a, that meet requirements to perform high complexity tests
- The ViroKey™ SARS-CoV-2 RT-PCR Test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens. The ViroKey™ SARS-CoV-2 RT-PCR Test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- The product is to be used by qualified and trained laboratory personnel only.
- The ViroKey™ SARS-CoV-2 RT-PCR Test is validated for use on specimens obtained from nasopharyngeal swabs from patients.
- Strict compliance with the instructions for use is required for optimal PCR results.
- Each tube of reagent is designed for 50 reactions.
- Do not use expired kit components. Expiration dates are printed on the box and labels of all components. RNA4 M3 is an enzyme, which is in liquid state. Except RNA4 M3, the rest of the ViroKey™ SARS-CoV-2 RT-PCR Test components should be thawed completely at room temperature (approximately 15°C – 25°C) for up to 30 minutes before use...
- RNA4 M3 should be used directly out of the freezer or kept on ice when performing reagent preparation. Handle carefully to avoid contamination and store the remaining RNA4 M3 immediately after use at ≤ −20°C for subsequent reactions.
- All reagents, except RNA4 M2 and RNA4 M3, require thorough mixing by quick vortex. Mix RNA4 M2 and RNA4 M3 by gentle inversion. Centrifuge all tubes briefly to collect the contents at the bottom of the tubes. Avoid foaming of the reagents.
- All relevant documents (refer to "Resources" section) should be read thoroughly before performing the assay.
- Mutations that arise within the highly conserved regions of the viral genome covered by the kit's primers and / or probes may

result in failure to detect the presence of the virus. May cause allergic skin reactions.

- May be harmful if swallowed.
- Use personal protective equipment as required.
- For additional information, please refer to the Material Safety Data Sheet (MSDS).
- All samples and waste should be considered potentially infectious. Clean and disinfect all work surfaces thoroughly with disinfectants recommended by local authorities.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection when handling samples and kit reagents.
- Clean and decontaminate work area and instruments, including pipettes, with commercially available decontamination products.
- Avoid microbial and nuclease contamination of reagents when removing aliquots from reagent bottles. Use sterile disposable pipette tips.
- To avoid environmental contamination by amplicons, do not remove the PCR seal after amplification.
- Wash hands thoroughly after handling biological samples and kit reagents.

## Regulatory status

This test is for use under an Emergency Use Authorization.
For Prescription Use Only.

## Symbols

| Symbol | Description |
|---|---|
|  | Contains reagents sufficient for <*n*> tests |
|  | Use-by date |
|  | For *in vitro* diagnostic (IVD) use |
|  | Prescription device |
|  | Catalog number |
|  | Component |
|  | Number |
|  | Content |
|  | Lot number |
|  | Control |
|  | Negative control |
|  | Positive control |
|  | Document / label identification number |
|  | Temperature limitations |
|  | Legal manufacturer |
|  | Refer to instructions for use |

## Resources

The latest edition of the MSDS and instructions for use of ViroKey™ SARS-CoV-2 RT-PCR Test and *Sentosa*® SX Virus Total Nucleic Acid Kit (4x24) v2.0 are available for download at www.veladx.com by logging in as an authorized user, or requesting them via email.



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## Contacts

**Manufacturer**
Vela Operations Singapore Pte Ltd
(Co Reg No. 201106974H)
50 Science Park Road
#05-07 The Kendall
Singapore 117406
Tel: +65 6672 6060
Fax: +65 6672 6066
General inquiry email: infoAPAC@veladx.com

**Distributor**
Vela Diagnostics USA Inc.
353C Route 46 West
Suite 250
Fairfield, NJ 07004
Orders: +1 877 593 7528
Technical: +1 877 593 7528
Fax: +1 973 521 7077
General inquiry email: infoUSA@veladx.com

**Regional service and support**
United States of America: support.us@veladx.com

**Visit our website:** www.veladx.com