

ViroKey[®] HT SARS-CoV-2 RT-PCR Test

Instructions for Use

For use under an Emergency Use Authorization Only

For Prescription Use Only

For In Vitro Diagnostic Use





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



301085 & 301088



8x48 tests



PS104064A



Consult instructions for use

Version 1.0

Contents

Kit contents	1
Symbols	2
Storage	3
Intended use	4
Warnings and Precautions	5
Safety information	6
Quality control	6
Introduction	7
Principle	7
Pathogen information	8
Limitations	
Conditions of Authorization for the Laboratory	9
Controls	
Result interpretation	11
Performance characteristics	13
Analytical sensitivity	
Analytical reactivity and specificity	13
Clinical performance	20
Workflow	20
Items to be supplied by user	22
Important notes	23
General precautions	
Protocol: Hamilton Microlab [®] STAR [™] automated workflow	24
Important points before starting	
ViroKey [®] HT Virus Total Nucleic Acid Kit	
Sample preparation	
Fresh samples	25
Samples stored at 4°C	25
Frozen samples	25
Froz ['] en samples Sample Tube Criteria for Hamilton Microlab[®] STAR[™] instrument 1. Automated nucleic acid extraction and RT-PCR set up on the Hamilton Microlab[®] STAR[™]	20
instrument	28
PCR and data analysis using the Sentosa [®] SA201 Real-Time PCR Instrument and	
Sentosa [®] SA201 Reporter software 2. PCR on the Sentosa [®] SA201 Real-Time PCR Instrument	4/
 PCR on the Sentosa SA201 Real-Time PCR Instrument	
Instrument maintenance	
Troubleshooting guide	65

Appendix for Sample Plate Preparation	. 67
Appendix for Viral RNA Extraction	.71
Appendix for HT PCR setup	.77
References	. 81
Contacts	. 83

Kit contents

ViroKey[®] HT SARS-CoV-2 RT-PCR Test Kit

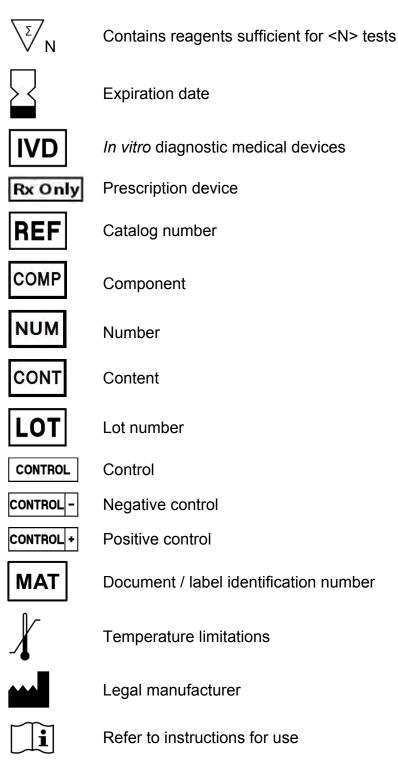
Kit item no.	ltem	Cap color	Description	Quantity	Amount
	HT SARS-CoV-2 M1	Green	Mix 1	4	115 µL
	HT RNA M2	Yellow	Mix 2	4	1400 µL
301085	HT RNA M3	Blue	Mix 3	4	112 µL
301065	HT NC	Natural	Negative control (NC)	4	300 µL
	HT SARS-CoV-2 PC	Blue	Positive control (PC)	4	300 µL
	HT EC	Red	Extraction control (EC)	4	1200 µL

ViroKey[®] HT Virus Total Nucleic Acid Kit

Kit item no.	ltem	Cap color	Description	Quantity	Amount
	Buffer D1	Natural	Buffer D1	4	80 mL
	Buffer D2	Natural	Buffer D2	4	80 mL
	Buffer D3	Natural	Buffer D3	4	25 mL
301088	Buffer D4	Natural	RNase-free water	4	25 mL
	Mag Beads	White	Magnetic bead suspension	4	2.8 mL
	cRNA	Red	Carrier RNA	4	310 µg

For Prescription Use Only

Symbols



For Prescription Use Only

Storage

The components of the ViroKey[®] HT SARS-CoV-2 RT-PCR Test should be stored at $-20^{\circ}C \pm 5^{\circ}C$ and are stable until the expiration date stated on the label. HT RNA M3 is an enzyme, which is in liquid state. Except HT RNA M3, all reagents should be thawed completely before use. All reagents should not be subjected to more than five (5) repeated freeze-thaw cycles as this may compromise assay performance.

Kit item no.	ltem	Quantity	Volume / tube	Shipping Condition	Storage Condition
	HT SARS-CoV-2 M1	4	115 µL	Dry ice	-25°C – -15°C
	HT RNA M2	4	1400 µL	Dry ice	-25°C – -15°C
301085	HT RNA M3	4	115 µL	Dry ice	-25°C – -15°C
301065	HT NC	4	300 µL	Dry ice	-25°C – -15°C
	HT SARS-CoV-2 PC	4	300 µL	Dry ice	-25°C – -15°C
	HT EC	4	1200 µL	Dry ice	-25°C – -15°C

ViroKey[®] HT SARS-CoV-2 RT-PCR Test Kit

ViroKey[®] HT Virus Total Nucleic Acid Kit

Kit item no.	ltem	Quantity	Volume / tube	Shipping Condition	Storage Condition
	Buffer D1	4	80 mL	Room Temperature	15°C – 25°C
	Buffer D2	4	80 mL	Room Temperature	15°C – 25°C
301088	Buffer D3	4	25 mL	Room Temperature	15°C – 25°C
301000	Buffer D4	4	25 mL	Room Temperature	15°C – 25°C
	Mag Beads	4	2.8 mL	Room Temperature	15°C – 25°C
	cRNA	4	310 µg	Room Temperature	15°C – 25°C

For Prescription Use Only

Intended use

ViroKey[®] HT SARS-CoV-2 RT-PCR Test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal or nasopharyngeal aspirates, nasal washes and bronchoalveolar lavage samples 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 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 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[®] HT 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[®] HT SARS-CoV-2 RT-PCR Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

For Prescription Use Only

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[®] HT 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[®] HT 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[®] HT 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 Federal Food, Drug, and Cosmetic 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 96 reactions.
- Do not use expired kit components. Expiration dates are printed on the box and labels of all components. HT RNA M3 is an enzyme, which is in liquid state. Except HT RNA M3, the rest of the ViroKey[®] HT 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.
- HT RNA 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 HT RNA M3 immediately after use at ≤ -20°C for subsequent reactions.
- All reagents, except HT RNA M2 and HT RNA M3, require thorough mixing by quick vortex. Mix HT RNA M2 and HT RNA 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.

For Prescription Use Only

- 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 and mask. For more information on the ViroKey[®] HT Virus Total Nucleic Acid Kit and the ViroKey[®] HT 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[®] HT SARS-CoV-2 RT-PCR Test is tested against predetermined specifications to ensure consistent product quality.

For Prescription Use Only

Introduction

The ViroKey[®] HT SARS-CoV-2 RT-PCR Test comprises a ready-to-use system for the detection of SARS-CoV-2 RNA extracted with the ViroKey[®] HT Virus Total Nucleic Acid Kit on the Hamilton Microlab[®] STARTM. SARS-CoV-2 RNA detection is done via reverse transcription PCR (RT-PCR) on the *Sentosa[®]* SA201 Real-Time PCR Instrument (hereinafter known as *Sentosa[®]* SA201).

The ViroKey[®] HT SARS-CoV-2 RT-PCR Test has specific primers and probes that target the *ORF1a* (FAM reporter dye) and *N* (HEX reporter dye) gene sequences of RNA from SARS-CoV-2 virus for direct detection of the specific amplicons in the same RT-PCR reaction. The genes are detected in the green and orange fluorescence channels respectively, on the *Sentosa*[®] SA201 Real-Time PCR System.

In addition, the ViroKey[®] HT SARS-CoV-2 RT-PCR Test contains a third set of primers and probe designed to detect an extraction control (EC) target in the red fluorescence channel. This extraction control is a non-human synthetic DNA fragment added to all samples to control for the nucleic acid extraction steps, and also function 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 (HT NC) and a positive control (HT SARS-CoV-2 PC) that allows the user to assess whether the RT-PCR reaction performed correctly.

Principle

The ViroKey[®] HT SARS-CoV-2 RT-PCR Test uses TaqMan[®] probe chemistry for realtime RT-PCR detection of viral nucleic acid extracted from respiratory specimens using the ViroKey[®] HT Virus Total Nucleic Acid Kit on Sentosa[®] automated workflow using the liquid handler Hamilton Microlab[®] STARTM instrument. The ViroKey[®] HT SARS-CoV-2 RT-PCR Test contains two primer/probe sets that target the *ORF1a* (FAM reporter dye in the Green fluorescence channel) or *N* (HEX reporter dye in the Orange fluorescence channel) gene sequences of RNA from SARS-CoV-2 virus. The assay also contains primers and a probe to detect an extraction control (EC) sequence, which is a nonhuman synthetic DNA fragment added to each sample (reporter dye in the Red fluorescence channel).

Nucleic acids extracted from specimens are reverse transcribed into cDNA, and cDNA sequences that are complementary to the oligonucleotide primers are amplified by polymerase chain reaction using the supplied enzyme mixes (tubes HT RNA M2 and HT RNA M3) on the *Sentosa*[®] SA201 Real-Time PCR system with the *Sentosa*[®] SA201 Reporter software. If the target nucleic acids are present and amplified, the probe(s) will anneal to specific complementary sequences located between the corresponding forward and reverse primers during the PCR process. During the extension phase of the PCR, the 5' nuclease activity of DNA polymerase degrades the probe bound to the specific target, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. Probes specific to each target generate a fluorescent signal at different wavelengths, enabling the instrument to differentiate between the signals. With

For Prescription Use Only

each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the *Sentosa*[®] SA201 Real-Time PCR system with the *Sentosa*[®] SA201 Reporter software. Real time monitoring of fluorescence intensities during PCR run allows the detection of the accumulating product¹.

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 upper respiratory tract infections (e.g. common cold) in humans². Other symptoms include rhinitis, cough, sore throat, and fever³.

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)⁴. 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 confirmed⁵. In February 2020, the International Committee on Taxonomy of Viruses named the novel coronavirus SARS-CoV-2⁶.

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[®] HT 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[®] HT SARS-CoV-2 RT-PCR Test was validated with nasopharyngeal swabs. Nasal (self-collected under supervision of, or healthcare provider collected), mid-turbinate, and oropharyngeal swab specimens as well as nasopharyngeal wash/aspirate or nasal aspirate specimens, as well as bronchoalveolar lavage samples are also considered acceptable specimen types, but performance has not been established.
- The ViroKey[®] HT SARS-CoV-2 RT-PCR Test has not been evaluated for patients receiving intranasally administered influenza vaccine.
- 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

For Prescription Use Only

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 virus.
- 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[®] HT 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-19emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.
- However, to assist clinical laboratories using the ViroKey[®] HT SARS-CoV-2 RT-PCR Test ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:
 - (A) Authorized laboratoriesⁱ 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.

For Prescription Use Only

- (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 permitted
- (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) 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.
- (G) Vela Diagnostics, authorized distributors, and authorized laboratories using ViroKey[®] HT SARS-CoV-2 RT-PCR Test 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.

ⁱ 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 for each run.

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 *N* gene, is included in the kit for each run.

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[®] HT 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.

Result interpretation

Negativity / positivity

The Ct ranges to define negativity / positivity for negative control, positive control and samples for the workflows are listed in the table below. If Ct falls within the range, it is defined as positive (+); if Ct falls out of the range or no Ct is obtained, it is defined as negative (-).

	Expected Ct values								
Fluorescence	Negative	e control	Positive	e control	Samples				
channel	Not detected	Detected	Not detected	Detected	Not detected	Detected			
Croon	< 10.0,		< 15.0,		< 10.0,				
Green (<i>ORF1a</i> gene)	> 40.0	10.0 – 40.0	> 32.0	15.0 – 32.0	> 40.0	10.0 - 40.0			
	or no Ct		or no Ct		or no Ct				
Orango	< 10.0,		< 15.0,		< 10.0,				
Orange (N gene)	> 40.0	10.0 – 40.0	> 32.0	15.0 – 32.0	> 40.0	10.0 - 40.0			
(N gene)	or no Ct		or no Ct		or no Ct				
Bod (avtraction	< 23.0,		< 23.0,		< 20.0,				
Red (extraction control, EC)	> 32.0	23.0 – 32.0	> 32.0	23.0 – 32.0	> 40.0	20.0 - 40.0			
	or no Ct		or no Ct		or no Ct				

For Prescription Use Only

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.

	Green (<i>ORF1a</i>)	Orange (<i>N</i>)	Red (EC)	Interpretation			
	-	-	+	Run valid (proceed to PC)			
Nevertier	+	-	+/-				
Negative control	-	+	+/-	Run invalid. Repeat run			
control	+	+	+/-	Run invalid. Repeat run.			
	-	-	-				
	+	+	+/-	Run valid (proceed to SARS-CoV-2 positive sample control)			
		-	+/-				
control	-	+	+/-	Run invalid. Repeat run.			
	-	-	+/-				
	+	+	+/-	Run valid (proceed to result interpretation of samples)			
SARS-CoV-2 positive	+	-	+/-				
sample	-	+	+/-	Run invalid. Repeat run.			
	-	-	+/-				
	+	+	+/-				
	-	+	+/-	SARS-CoV-2 virus detected*			
Samples	+	-	+/-				
-		-	+	SARS-CoV-2 virus not detected			
	-	-	-	Sample invalid. Sample should be retested. If 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

For Prescription Use Only

Performance characteristics

Analytical sensitivity

The analytical limit of detection (LoD) of ViroKey[®] HT SARS-CoV-2 RT-PCR Test was assessed with the *Sentosa*[®] workflow, starting with sample extraction using the ViroKey[®] HT Virus Total Nucleic Acid Kit on the Hamilton Microlab[®] STARTM, as well as RT-PCR run on the *Sentosa*[®] SA201. Serial dilutions of the heat-inactivated 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 1.0 x 10³ and 125 (GE/mL). 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 was obtained. The overall assay LoD was the lowest dilution giving a final sample detection of \ge 95% for 20 samples for one of the targets. (**Table 1**). The overall LoD of the ViroKey[®] HT SARS-CoV-2 RT-PCR Test is 200 GE/mL.

Table 1. Results from confirmatory LoD study for the ViroKey [®] HT SARS-CoV-2
RT-PCR Test.

Target channel	Genomic equivalents/mL	Detection %	Mean Ct ± SD
ORF1a (Green channel)	187.5	95% (19/20)	34.02 ± 1.13
N (Orange channel)	200	100% (20/20)	33.88 ± 0.57

Analytical reactivity and specificity

The analytical reactivity and specificity of the ViroKey[®] HT 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[®] HT SARS-CoV-2 RT-PCR Test for SARS-CoV-2, *in silico* analysis was performed on all sequences available on the National Center for Biotechnology Information (NCBI) GenBank and Global Initiative on Sharing All Influenza Data (GISAID) databases. 2,636,603 complete sequences (401,846 from NCBI GenBank and 2,234,757 from GISAID — sequences downloaded on 4th August 2021) were aligned against ViroKey[®] HT SARS-CoV-2 RT-PCR Test primers and probes. The sequences were aligned with MAFFT (https://mafft.cbrc.jp/alignment/server/).

Out of the 401,846 complete SARS-CoV-2 sequences from NCBI GenBank database as of 4th August 2021,

• *ORF1a* primers and probes had 100% match to 396,292 out of 401,846 sequences

For Prescription Use Only

(98.61%). For the remaining 5554 sequences (1.39%), the primers and probes have at most 1 mismatch with these sequences.

• *N* gene primers and probes had 100% match to 390,973 out of 401,846 sequences (97.29%). For the remaining 10,873 sequences (2.71%), the primers and probes have at most 1 mismatch with these sequences.

Out of the 2,234,757 complete SARS-CoV-2 sequences from GISAID database as of 4th August 2021,

- *ORF1a* primers and probes had 100% match to 2,202,254 out of 2,234,757 sequences (98.54%). For the remaining 32,503 sequences (1.46%), the primers and probes have at most 1 mismatch with these sequences.
- *N* gene primers and probes had 100% match to 2,163,247, out of 2,234,757 sequences (96.87%). For the remaining 71,510 sequences (3.2%), the primers and probes have at most 1 mismatch with these sequences.

In silico analysis concluded that ViroKey[®] HT SARS-CoV-2 RT-PCR Test will detect all analysed SARS-CoV-2 sequences in the NCBI GenBank (n = 401,846) and in GISAID (n = 2,234,757) databases. 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[®] HT SARS-CoV-2 RT-PCR Test, *in silico* analysis was performed on pathogens listed in **Table 2**. NCBI BLAST tool was used to check for cross-reactivity of the different primers and probes of the ViroKey[®] HT 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.

		In silico analysis for % identity/homology					у	
			N ORF1a					
Microorganism	Genbank Acc 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	No aligni	No alignment was found			No alignment was found		
Coronavirus HKU-1	NC_006577.2	No aligni	No alignment was found			No alignment was found		
Coronavirus NL63	NC_005831.2	No aligni	ment was	found	No alignment was found			
SARS-coronavirus	NC_004718.3	NA	NA	70%	No alignr	nent was t	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 aligni	ment was	found	No alignr	No alignment was found		

Table 2. *In silico* analysis for ViroKey[®] HT SARS-CoV-2 RT-PCR Test.

For Prescription Use Only

		In silico analysis for % identity/homology					у
			N		(ORF1a	
Microorganism	Genbank Acc No.	Forward Primer	Reverse Primer	Probe	Forward Primer	Reverse Primer	Probe
Human adenovirus 54	NC_012959.1	65%	NA	NA	No alignn	nent was	found
Human adenovirus A	NC_001460.1	No alignr	nent was	found	No alignn	nent was i	found
Human adenovirus B1	NC_011203.1	No alignr	ment was	found	No alignn	nent was i	found
Human adenovirus B2	NC_011202.1	No alignr	nent was	found	No alignn	nent was t	found
Human adenovirus C	NC_001405.1	No alignr	nent was	found	No alignn	nent was i	found
Human adenovirus D	NC_010956.1	No alignr	nent was	found	No alignn	nent was i	found
Human adenovirus E	NC_003266.2	No alignr	nent was	found	No alignn	nent was i	found
Human adenovirus F	NC_001454.1	No alignr	nent was	found	No alignn	nent was t	found
Human adenovirus type 1	AC_000017.1	No alignr	nent was	found	No alignn	nent was i	found
Human adenovirus type 35	AC_000019.1	No alignr	nent was	found	No alignn	nent was i	found
Human adenovirus type 7	AC_000018.1	No alignr	nent was	found	No alignn	nent was i	found
Human metapneumovirus	NC_039199.1	No alignr	nent was	found	No alignn	nent was i	found
Human parainfluenza virus 1	NC_003461.1	No alignr	nent was	found	No alignn	nent was i	found
Human parainfluenza virus 2	NC_003443.1	No alignr	ment was	found	No alignn	nent was i	found
Human parainfluenza virus 3	NC_001796.2	No alignr	ment was	found	No alignn	nent was i	found
Human parainfluenza virus 4a	NC_021928.1	No alignr	ment was	found	No alignn	nent was i	found
Influenza A virus H1N1	GCF_000865725	No alignr	nent was	found	No alignn	nent was i	found
Influenza A virus H3N2	GCF_000865085	No alignr	nent was	found	No alignn	nent was i	found
Influenza A virus H5N1	GCF_000864105	No alignr	nent was	found	No alignn	nent was i	found
Influenza A virus H7N9	GCF_000928555	No alignr	ment was	found	No alignn	nent was i	found
Influenza B virus	GCF_000820495	No alignr	nent was	found	No alignn	nent was i	found
Influenza C virus	GCF_000856665. 10	No alignr	ment was	found	No alignn	nent was	found
Human Parechovirus	NC_001897.1	No alignr	nent was	found	No alignment was found		
Enterovirus (e.g. EV68)	NC_038308.1	No alignr	nent was	found	No alignment was found		
Human respiratory syncytial virus	NC_001781.1	No alignr	ment was	found	No alignment was found		
Human rhinovirus 1	NC_038311.1	No alignr	ment was	found	No alignn	nent was i	found
Human rhinovirus 3	NC_038312.1	No alignr	ment was	found	No alignn	nent was i	found
Human rhinovirus 14	NC_001490.1	No alignr	ment was	found	No alignn	nent was i	found
Human rhinovirus 89	NC_001617.1	No alignr	ment was	found	No alignn	nent was i	found
Human rhinovirus C	NC_009996.1	No alignr	ment was	found	No alignn	nent was i	found
Chlamydophila pneumoniae	NC_002180.1	No alignr	nent was	found	No alignn	nent was	found
Haemophilus influenzae	NZ_LN831035.1	65%	NA	NA	NA	NA	60%
Legionella pneumophila	NZ_LR134380.1	65%	68%	NA	67%	NA	56%
Mycobacterium tuberculosis	NC_000962.3		ment was	r	67%	NA	NA
Streptococcus pneumonia*	NZ_LN831051.1	80%	68%	NA	NA	NA	52%
Streptococcus pyogenes	NC_002737.2	No alignr	ment was	found	NA	NA	60%

For Prescription Use Only

		In silico analysis for % identity/homology					
			N		(ORF1a	
Microorganism	Genbank Acc No.	Forward Primer	Reverse Primer	Probe	Forward Primer	Reverse Primer	Probe
Bordetella pertussis	NC_018518.1	65%	NA	NA	62%	NA	NA
Mycoplasma pneumoniae	NZ_CP010546.1	No aligni	ment was	found	NA	NA	52%
Pneumocystis jirovecii (PJP)	GCF_001477535. 1	No aligni	ment was	found	NA	NA	52%
Candida albicans*	GCF_000182965. 3	65%	74%	67%	62%	NA	60%
Pseudomonas aeruginosa	GCF_000006765. 1	65%	NA	NA	67%	NA	52%
Staphylococcus epidermis	GCF_000007645. 1	NA	68%	NA	NA	71%	NA
Staphylococcus salivarius	CP013216.1	NA	74%	NA	No alignr	ment was	found
Leptospira borgpetersenii	GCF_000013945	65%	NA	NA	NA	NA	56%
Leptospira interrogans	GCF_000092565	65%	79%	NA	No alignment was four		found
Leptospira santarosai*	GCF_000313175	70%	89%	NA	NA	62%	NA
Chlamydia psittaci	NC_017287.1	No aligni	ment was	found	No alignment was four		
Coxiella burnetii (Q-Fever)	NC_002971.4	NA	68%	NA	62%	NA	60%
Staphylococcus aureus	NC_007795.1	70%	68%	NA	No alignment was found		found
Klebsiella pneumonia*	GCF_000240185. 1	80%	84%	NA	62%	NA	52%
Corynebacterium diphtheriae	NZ_LN831026.1	65%	68%	NA	NA	NA	56%
Legionella longbeachae*	GCF_000091785. 1	65%	68%	NA	81%	67%	56%
Bacillus anthracosis (Anthrax)	GCF_000008445. 1	NA	68%	NA	NA	NA	56%
Moraxella catarrhalis	NC_014147.1	85%	NA	NA	NA	62%	52%
Neisseria elongata	NZ_CP007726.1	70%	68%	NA	62%	NA	NA
Neisseria meningitidis	NZ_LR134525.1	65%	68%	NA	62%	NA	52%
Pooled human nasal wash - to represent diverse microbial flora in the human respiratory tract*	ZLYEM2C (HMP)	80%	74%	67%	86%	71%	60%
Bat coronavirus HKU4-1	NC_009019.1	No aligni	ment was	found	48%	NA	40%
Bat coronavirus HKU5-1	NC_009020.1	No aligni	ment was	found	NA	48%	NA
Bat coronavirus HKU9-1	NC_009021.1	No aligni	ment was	found	NA	48%	NA
Scotophilus bat coronavirus 512	NC_009657.1	No alignment was found			NA	NA	40%
Bat coronavirus HKU2	NC_009988.1	No aligni	ment was	found	48%	48%	48%
Bat coronavirus 1A	NC_010437.1	No aligni	ment was	found	48%	48%	NA
Bat coronavirus HKU8	NC_010438.1	•	ment was		NA	NA	40%
Bat coronavirus BM48-	NC_014470.1	No aligni	ment was	found	NA	57%	NA

		In silico analysis for % identity/homology				у	
			N		(ORF1a	
Microorganism	Genbank Acc No.	Forward Primer	Reverse Primer	Probe	Forward Primer	Reverse Primer	Probe
31/BGR/2008							
Rousettus bat coronavirus HKU10	NC_018871.1	No align	ment was	found	48%	NA	40%
Bat coronavirus CDPHE15/USA/2006	NC_022103.1	No align	ment was	found	NA	48%	40%
BtMr-AlphaCoV/SAX2011	NC_028811.1	No alignment was found		NA	48%	48%	
BtRf-AlphaCoV/HuB2013	NC_028814.1	No align	ment was	found	NA	48%	NA
BtRf-AlphaCoV/YN2012	NC_028824.1	No align	ment was	found	NA	57%	48%
BtNv-AlphaCoV/SC2013	NC_028833.1	No align	ment was	found	NA	48%	40%
Rousettus bat coronavirus isolate GCCD.1 356	NC_030886.1	No alignment was found		NA	48%	NA	
NL63-related bat coronavirus strain BtKYNL63-9a	NC_032107.1	No alignment was found		NA	NA	40%	
Bat coronavirus isolate PREDICT/PDF-2180	NC_034440.1	No align	ment was	found	NA	NA	40%

For Prescription Use Only

Some forward primers, reverse primers, or probes sequences have significant alignments (>80%) with the sequences and these are highlighted in red in table above. Among those are *Streptococcus pneumonia, Leptospira santarosai, Klebsiella pneumonia, Legionella longbeachae and Moraxella catarrhalis*. These pathogens were further analyzed and no potential cross-reactivity 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 out a PCR product that can be detected on the ViroKey[®] HT SARS-CoV-2 RT-PCR Test. *In silico* analysis of pooled microflora showed potential for cross-reactivity, therefore wet testing was performed (described below).

Analytical specificity (wet testing)

The ViroKey[®] HT 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[®] HT SARS-CoV-2 RT-PCR Test PCR mix. At least three replicates were tested. All controls performed as expected. The results are presented in **Table 3**.

Table 3. Potential cross-reactivity of the ViroKey[®] HT SARS-CoV-2 RT-PCR Test.

	Tested pathogens, strain (RNA)	Sample input	ORF1a	N	EC
	Haemophilus influenzae		0/3	0/3	3/3
	Mycobacterium tuberculosis, H37Ra		0/3	0/3	3/3
	Streptococcus pneumoniae		0/3	0/3	3/3
Bacteria	Streptococcus pyogenes Rosenbach		0/3	0/3	3/3
Dacteria	Mycoplasma pneumoniae		0/3	0/3	3/3
	Legionella pneumophila		0/3	0/3	3/3
	Chlamydophila pneumoniae strain CM-1		0/3	0/3	3/3
	Bordetella pertussis		0/3	0/3	3/3
	Human coronavirus 229E		0/3	0/3	3/3
	Human coronavirus OC43		0/3	0/3	3/3
	Human coronavirus HKU1	5x 10 ⁶ copies	0/3	0/3	3/3
	Human coronavirus NL63	(1x 10 ⁶	0/3	0/3	3/3
	Human metapneumovirus (hMPV)	copies/	0/3	0/3	3/3
	Human adenovirus 1, Adenoid 71	reaction)	0/3	0/3	3/3
	Human parainfluenza virus 2, Greer		0/3	0/3	3/3
Virus	Human parainfluenza virus 3, C243		0/3	0/3	3/3
VIIUS	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, A/Cali/07/2009 (H1N1 pdm)		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
Natural h	uman flora – pooled human nasal wash	NA	0/3	0/3	3/3

For Prescription Use Only

The ViroKey[®] HT SARS-CoV-2 RT-PCR Test was also evaluated for ability to detect the SARS-CoV-2 variants of concerns at 3x LoD across three replicates The results are presented in **Table 4**.

Table 4. Reactivity of the ViroKey[®] HT SARS-CoV-2 RT-PCR Test.

SARS-CoV-2 Variants tested	Sample input	ORF1a	N
Alpha B.1.1.7 variant (Twist Bioscience RNA Control 15)		32.75 ± 1.10	32.46 ± 0.31
Beta B.1.351 variant (Twist Bioscience RNA Control 16)	3x LoD	32.38 ± 0.26	31.92 ± 0.25
Gamma P.1 variant (Twist Bioscience RNA Control 17)	3X LOD	32.29 ± 0.06	32.83 ± 0.28
Delta B.1.617.2 variant (Twist Bioscience RNA Control 23)		31.12 ± 0.05	31.50 ± 0.56

Interfering substances

For Prescription Use Only

The objective of the study was to verify the effect of potentially interfering substances on the performance of ViroKey[®] HT SARS-CoV-2 RT-PCR Test. Base pool of SARS-CoV-2 positive samples were prepared with heat-inactivated SARS-CoV-2 virus from American Type Culture Collection (ATCC[®] part number VR-1986HK) 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 the specified test concentrations noted in **Table 5** for each interference substance. This study demonstrates that the potential interfering substances tested at specific concentrations as stated in **Table 5** have no impact on the performance of ViroKey[®] HT SARS-CoV-2 RT-PCR Test.

Table 5. List of potential interferin	g substances	tested wit	h ViroKey [®]	HT SARS-
CoV-				
2 RT-PCR Test.				

Substance	Active Ingredient/s	Conc.	Detection % (ORF1a)	Detection % (N)
Nasal Wash (Flo [®])	Sodium chloride, potassium chloride, calcium lactate pentahydrate	15% (v/v)	100% (3/3)	100% (3/3)
Nasal Spray/drops (Nazolin [®])	Oxymetazoline HCI	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	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		2% (v/v)	100% (3/3)	100% (3/3)
Mucin	N.A.	60 µg/mL	100% (3/3)	100% (3/3)
Pooled human nasal wash		N.A.	100% (3/3)	100% (3/3)

FLUMIST nasal spray flu vaccine was not tested for its potential interference with ViroKey[®] HT SARS-CoV-2 RT-PCR Test.

For Prescription Use Only

Clinical performance

The clinical validation study of ViroKey[®] HT SARS-CoV-2 RT-PCR Test was conducted on nasopharyngeal swabs from unaltered patient samples initially tested with an FDA authorized molecular assay as a comparator method. All samples were extracted with ViroKey[®] HT Virus Total Nucleic Acid Kit on the Hamilton Microlab[®] STAR[™] instrument and detected with ViroKey[®] HT SARS-CoV-2 RT-PCR Test on the *Sentosa[®]* SA201.

A total of 68 nasopharyngeal samples were tested and the results are summarized in **Table 6**. The performance of the ViroKey[®] HT SARS-CoV-2 RT-PCR Test was calculated based on the result interpretation table on page 11, where a sample was considered positive if signals are detected by ORF1a and/or N target(s), while a sample was considered negative if no signals are detected by both ORF1a and N targets.

Table 6. Summary of clinical performance results.

		Comparat	tor Results
		Positive	Negative
ViroKey [®] HT SARS-CoV-2	Positive	30	0
RT-PCR Test	Negative	0	30

Positive Agreement: 100% (30/30) 95% CI: 88.6 - 100% Negative Agreement: 100% (30/30) 95% CI: 88.6 - 100%

Workflow

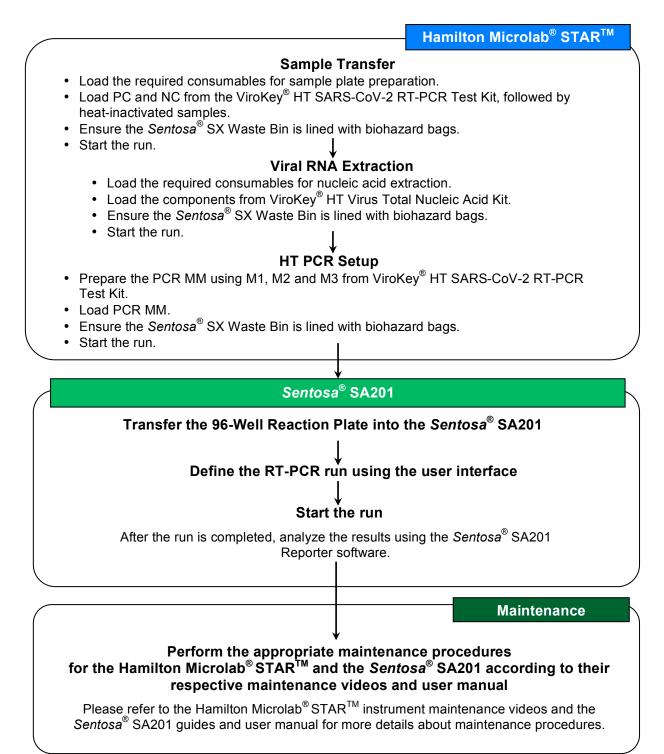
The workflow starts with sample plate preparation followed by the lysis and extraction of nucleic acids, and RT-PCR set up with the extracted nucleic acids in the MicroAmp[®] Fast Optical 96-Well Reaction Plate using the Hamilton Microlab[®] STARTM.

For nasopharyngeal swabs, use the ViroKey[®] HT Virus Total Nucleic Acid Kit for nucleic acid extraction.

After sample lysis and RT-PCR set-up, the MicroAmp[®] Fast Optical 96-Well Reaction is sealed, and then transferred to the *Sentosa[®]* SA201 for PCR amplification respectively. This is followed by data analysis using *Sentosa[®]* SA201 Reporter or SA Reporter.

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

Flowchart: Automated workflow overview



Items to be supplied by user

Table 1. List of items	to be se	upplied by	user.
------------------------	----------	------------	-------

Equipment / software	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
Thermomixer ⁱ	To heat and mix samples	N/A
Hamilton Microlab [®] STAR [™]	Automated sample processing system	400316
Sentosa [®] SA201 Real-Time PCR Instrument ⁱ	Real-time and end-point thermal cycling using PCR, detection and analysis	400125
Sentosa [®] SA201 Reporter ⁱ	To automate data analysis and result interpretation from <i>Sentosa</i> ® SA201	480142
Accessories / consumables / reagents	Description / use	Vela item no.
ViroKey [®] HT Virus Total Nucleic Acid Kit	4x96 tests	300678
HT 300 mL Reservoir (40)	To contain the reagents	400298
HT 8-Strip Tubes, Clear, 0.2 mL (125)	8-strip tubes (1 PCR Strip)	400299
HT Reagent Tub with Lid, 60 mL (28)	60 mL Trough	400307
HT U Deepwell Plate, Barcoded, 2.2 mL (24)	Deepwell plate	400308
HT Conductive 1 mL Filter Tips (3840)	For pipetting buffers, reagents and / or samples	400309
HT Conductive 300 µL Filter Tips (5760)	For pipetting buffers, reagents and / or samples	400310
HT Conductive 50 µL Filter Tips (5760)	For pipetting buffers, reagents and / or samples	400311
Sterile pipette tips with filters	For pipetting buffers, reagents and / or samples	N/A
MicroAmp [®] Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL ⁱⁱ	N/A	N/A
MicroAmp [®] Optical Adhesive Film ⁱⁱⁱ	N/A	N/A
MicroAmp [®] Adhesive Film Applicator ^v	For applying the MicroAmp [®] Optical Adhesive Film to seal the MicroAmp [®] Fast Optical 96-Well Reaction Plate with Barcode	N/A
Regular flocked swabs (nasopharyngeal) ^{vi}	For collecting nasopharyngeal swab samples	N/A
Absolute ethanol	For adding to Buffer D3 in ViroKey [®] HT Virus Total Nucleic Acid Kit	N/A

ⁱ Ensure that the instruments have been checked and calibrated according to the manufacturer's recommendations.

ⁱⁱ 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.

^{III} MicroAmp[®] Optical Adhesive Film from Applied Biosystems[®] (Cat. No. 4311971) **MUST** be used. Use only unexpired films.

^v MicroAmp[™] Adhesive Film Applicator from Thermo Fisher Scientific (Cat. No. 4333183) is recommended.

^{VI} BD[™] Regular flocked swab, sterile single wrapped (Cat. No. 220250) or BD[™] Flexible minitip flocked swab, sterile single wrapped (Cat. No. 220252) in BD[™] Universal viral transport, 3 mL vial (Cat. No. 220220) **OR** 16 X 100mm Screw Cap Tube containing 3 mL of UTM Transport and Preservation Medium, 1 Nasopharyngeal Flocked Swab (Cat. No. 305C).

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. Do not exceed 30 minutes of transfer time between the Hamilton Microlab[®] STAR[™] and the Sentosa[®] SA201.
- It is **not recommended** to store the remaining PCR reaction mix after PCR set-up.

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 \leq -70°C.

Protocol: Hamilton Microlab[®] STAR[™] automated workflow

The ViroKey[®] HT Virus Total Nucleic Acid Kit is intended for virus total nucleic acid extraction from respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens and nasopharyngeal wash/aspirate or nasal aspirate specimens) and bronchoalveolar lavage samples for use with the ViroKey[®] HT SARS-CoV-2 RT-PCR Test.

Important points before starting

- User must be familiar with operating the Hamilton Microlab[®] STAR[™] instrument and the *Sentosa*[®] SA201. Please refer to the respective user manuals supplied with the instruments for operating instructions.
- Before beginning the procedure, read the "Important notes" section, page 23.
- Ensure that all reagents of the ViroKey[®] HT Virus Total Nucleic Acid Kit are not precipitated before use. If precipitates are observed, dissolve by incubating in a water bath (≤ 37°C).

ViroKey[®] HT Virus Total Nucleic Acid Kit

- The Mag tubes containing magnetic beads require thorough vortexing for 5 minutes before the start of the workflow to ensure proper resuspension.
- Prior to use, cRNA (lyophilized carrier RNA) must be reconstituted. Refer to detailed procedure for more information.
- Mix the buffers in the bottles by gentle swirling, ensuring no foam or bubbles are present.
- All components should be used within 30 minutes after removal of caps.
- HT RNA M3 is an enzyme, which is in liquid state. Except HT RNA M3, the rest of the ViroKey[®] HT SARS-CoV-2 RT-PCR Test components should be thawed at room temperature (approximately 15°C – 25°C).
- For software, use current version or higher

Hamilton Run Control	Release 4.5.0.5217 (for use with Hamilton Microlab [®] STAR [™])
Sentosa [®] SA201 Series Software Sentosa [®] SA201 Reporter	Version 1.0.1 (for use with Sentosa [®] SA201) Version 1.6 (for use with Sentosa [®] SA201)

• Screenshots are for illustration purposes, and individual installations may vary.

Sample preparation

Sample in Universal Transport Medium (UTM) or Viral Transport Medium (VTM) tubes **must** be heat-inactivated at 75°C for 30 minutes before placing onto the Hamilton Microlab[®] STARTM instrument with the cap and swab removed for sample transfer⁷. Up to 376 samples, 4 positive control sample (PC) and 4 negative control (NC) sample can be performed in one run of the ViroKey[®] HT SARS-CoV-2 RT-PCR Test resulting in 4 PCR plates.

Fresh samples

- Vortex swab in Universal Transport Media for 30 seconds.
- Remove swabs from the tubes and discard the swabs according to the local safety regulations.
- Inactivate the SARS-CoV-2 virus in samples in an oven / a water bath at 75°C for 30 minutes.

Samples stored at 4°C

- Equilibrate to room temperature.
- Vortex swab in Universal Transport Media for 30 seconds.
- Remove swabs from the tubes and discard the swabs according to the local safety regulations.
- Inactivate the SARS-CoV-2 virus in samples in an oven / a water bath at 75°C for 30 minutes.

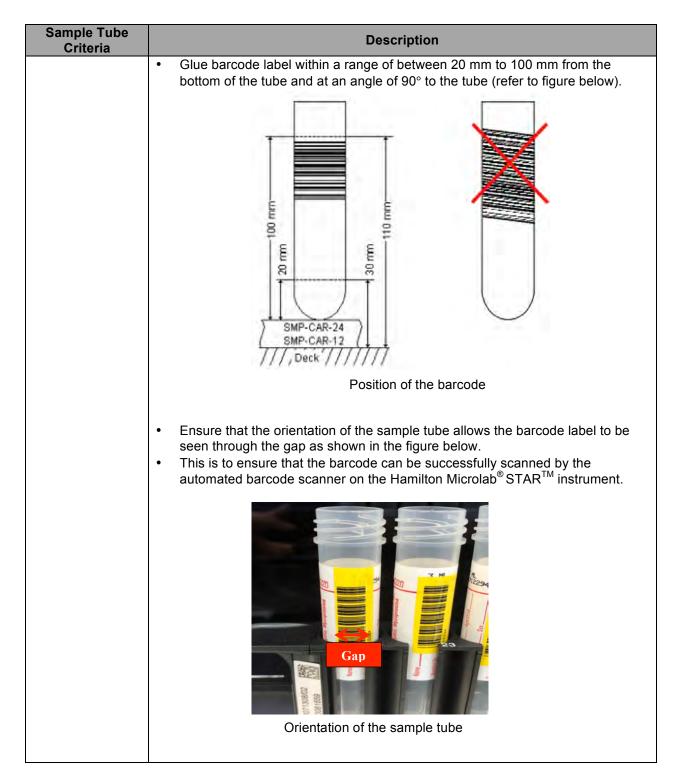
Frozen samples

- Thaw samples and equilibrate to room temperature.
- Vortex swab in Universal Transport Media for 30 seconds.
- Remove swabs from the tubes and discard the swabs according to the local safety regulations.
- Inactivate the SARS-CoV-2 virus in samples in an oven / a water bath at 75°C for 30 minutes.

Sample Tube Criteria for Hamilton Microlab[®] STAR[™] instrument

Sample Tube Criteria		Descri	ption			
Specifications						
Sample tube ID (Barcode)	• On sar	 Only alphanumeric characters are allowed (i.e. no symbols are allowed) for sample tube ID barcodes. 				
	bai • En	barcode label.				
Barcode labels		Label specifica	ations			
		Dimension	Minimum length	Maximum length		
	Α	Label length	N/A	80 mm		
	В	Code length	N/A	74 mm		
	с	Quiet zone	3 mm	N/A		
	D	Label width	12 mm	N/A		
	Е	Code width	12 mm	N/A		
	F	Distance from the barcode to the label edge	N/A	1 mm		

For Prescription Use Only



1. Automated nucleic acid extraction and RT-PCR set up on the Hamilton Microlab[®] STAR[™] instrument

The workflow on the Hamilton Microlab[®] STAR[™] instrument is split into three stages:

- Sample transfer,
- Viral RNA Extraction, and
- RT-PCR set up.

1.1. Sample transfer

Figure 1 shows the positions of consumables / labware on the Hamilton Microlab[®] STARTM platform. Double line the *w*aste bin with biohazard bags. Please refer to the layout as indicated by the Hamilton Microlab[®] STARTM instrument software or the appendix to load all items in the correct positions.

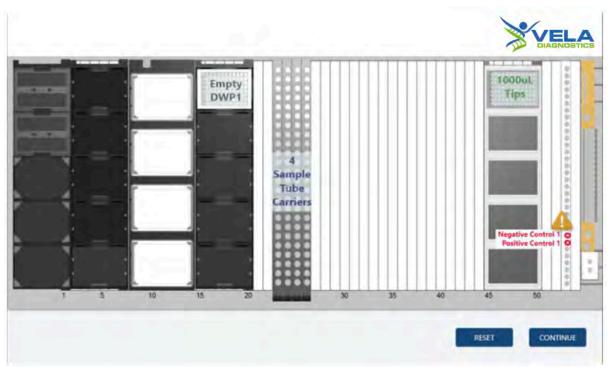


Figure 1. Layout of the Hamilton Microlab[®] STARTM platform for 1 deepwell plate (1x96 tests) for sample plate preparation (refer to Appendix for 2 to 4 deepwell plates layout).

NOTE:

- Items shown are necessary for nucleic acid extraction and PCR assay set-up for application *"STAR8AL96 Vela_SampleTransfer_V1.2.med"*.
- Ensure all consumables / labware are properly placed, aligned and secured into their respective positions.

For Prescription Use Only

- Ensure that the biohazard bags are properly attached to the waste bin before starting a protocol run. For more information, please refer to the Hamilton Microlab[®] STAR[™] instrument user manual.
- 1.1.1. Switch on the computer, and wait for the initialization procedure to be completed.
- 1.1.2. On the instrument's computer, launch the Hamilton Microlab[®] STAR[™]

software by double-clicking the 9 icon.

NOTE: Please switch on Hamilton Microlab[®] STARTM instrument after Hamilton Microlab[®] STARTM software is launched.

1.1.3. Press / Click "VIROKEY" button.

🧿 Hamilton Method Manager (Operator Only) - A	Ivaro Cuevas 2010		×
	Alvono Cuevos 2010 anoro our ve na presidore		
	Ŷ		
	VIROKEY		
Frequent Used Methods:		-	
2. Viral RNA Extraction	3, HT PCR Setup 1. Sample Plate Preparation		

1.1.4. Press / Click the "Run" under "1. Sample Plate Preparation" to launch the

For Prescription Use Only

MEASURE OF EXCELLENCE					_
	1. Sample Plate Preparation	() Run	Layout	Summary	close M Mex
Ŷ	2. Viral RNA Extraction	Run	Layout	Summary	Anex
VIROKEY	3. HT PCR Setup	Run	Layout	Summary	Anex
Frequent Used Methods:					
2. Viral RNA Extraction	3. HT PCR Setup	> 1. Sam	ple Plate Preparation	n > Independe	ant Channel Tip

sample plate preparation application.

1.1.5. Input value 1 to 4 (minimum 1 and maximum 4) for the *"NUMBER OF DEEPWELL PLATES"*. Load the empty deepwell plates onto the Hamilton Microlab[®] STAR[™] worktable (refer to **Figure 1** in step 1.1). Press / Click *"CONTINUE"* when done.

ViroKey HT Virus Total Nucleic Acid Kit	
NUMBER OF DEEPWELL PLATES:	1
	Min: 1 Max: 4
CANCEL	CONTINUE

1.1.6. Press / Click "CONTINUE" to proceed to scanning of NC and PC tubes.

For Prescription Use Only



Scan the 2D barcodes on the NC and PC for **Control Set 1 (NC1 and PC1)**. Load the **Control Set 1** onto **Tube Carrier Track 53**. Press / Click "*CONTINUE*" when done.



NOTE: Ensure that the NC and PC tubes are uncapped before loading.

For Prescription Use Only

1.1.7. [If applicable] Repeat step 1.1.6 for **Control Sets 2** to **4** if performing for 2x96 to 4x96 tests respectively.

NOTE: Each control set corresponds to each sample set in the table below. Each control set consists of 1 NC and 1 PC and each sample set consists of 94 samples.

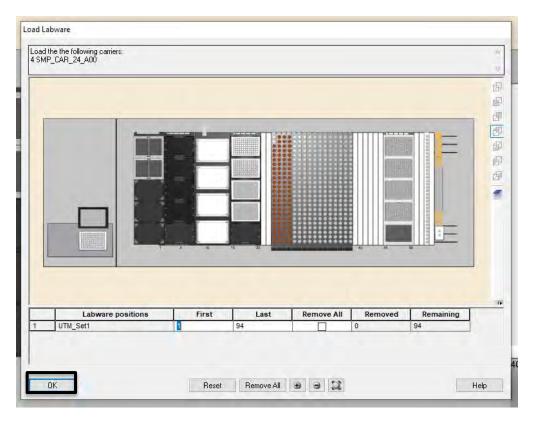
Track Carrier	Control Set	Sample Set
53	1	1
	2	2
	3	3
	4	4

1.1.8. Load the sample tubes onto the sample tube carriers.

Input the number of samples used under the *"First"* and *"Last"* columns or select the area where the *"First"* and *"Last"* samples are placed using the cursor (by *"clicking"* and *"dragging"* over an area). Press / Click *"OK"* when done.

NOTE:

- Ensure that the sample tubes are uncapped before loading.
- User can run less than 94 samples in each run by indicating the first and last sample in the carrier.
- After the user has indicated the *"First"* and *"Last"* samples, location of the samples are shown as "brown circles".



For Prescription Use Only

1.1.9. Press / Click *"Unload All Carriers"* after the run is completed. Unload all carriers, clean and disinfect the Hamilton Microlab[®] STAR[™] instrument after each run. For instrument maintenance, please refer to the Hamilton Microlab[®] STAR[™] instrument maintenance videos.



Proceed to "2. Viral RNA Extraction", on the "Hamilton Method Manager" user interface, after the run is completed.

1.2. Viral RNA Extraction

Figure 2 shows the positions of consumables / labware on the Hamilton Microlab[®] STARTM platform. Double line the *w*aste bin with biohazard bags. Please refer to the layout as indicated by the Hamilton Microlab[®] STARTM instrument software or the appendix to load all items in the correct positions.

000uL Tips			DWP1	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	Lysis
1000uL Tips									Beads
Buffer D2		C							00000
Buffer D3									000000000000000000000000000000000000000
	Buffer D4					1000uL Tips	50uL Tips		
1	5	10	15	20 25	30	35	Concernation of the local division of the lo	45 5	

Figure 2. Layout of the Hamilton Microlab[®] STAR[™] platform for 1 deepwell plate for viral RNA extraction (refer to Appendix for 2 to 4 deepwell plate layouts).

NOTE:

- Items shown are necessary for nucleic acid extraction for application "STAR8AL96 Vela_ViroKey_V3.10.med".
- Ensure all consumables / labware are properly placed, aligned and secured into their respective positions.
- Ensure that the biohazard bags are properly attached to the waste bin before starting a protocol run. For more information, please refer to the Hamilton Microlab[®] STARTM instrument user manual.

For Prescription Use Only

1.2.1. Press / Click the "RUN" under "2. Viral RNA Extraction".

AMILTON MEASURE OF EXCELLENCE	Alvaro Cuevas 2010				
MEASURE OF EXCELLENCE™					
					close (M
	1. Sample Plate Preparation	N un	Layout	Summary	Anex
Ŷ	2. Viral RNA Extraction	Run	Layout	Summary	Anex
VIROKEY	3. HT PCR Setup	Run	Layout	Summary	Anex
Frequent Used Methods:					
2. Viral RNA Extraction	3. HT PCR Setup	1. Samp	ole Plate Preparation		dent Channel Tip Transfer

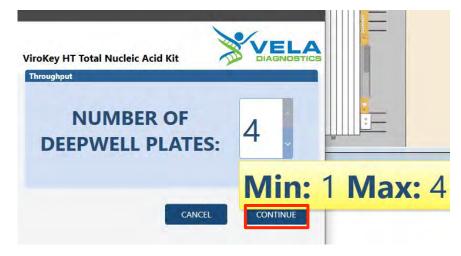
1.2.2. Empty the liquid waste bottle. Press / Click "DONE" after placing back the empty waste bottle.



For Prescription Use Only

1.2.3. Input value 1 to 4 (minimum 1; maximum 4) for the "NUMBER OF DEEPWELL

PLATES". Press / Click "CONTINUE" when done.



1.2.4. Prepare the reagents and consumables (Buffer D2 to D4, lysis master mix, 1,000 μ L tips and 50 μ L tips and beads). Press / Click *"CONTINUE"* when done.

Refer to Appendix for Consumables Summary for 2 to 4 deepwell plates.

-	1mL Tips	50uL Tips	2.2mL 96-well DWP	60mL Trough	300mL Trough
LABWARE			0	2	0
QTY	8	1	1	2 Lysis 40 ml Beads 40 ml	B uffer D2 80 m Buffer D3 125 m Buffer D4 25 m

For Prescription Use Only

1.2.5. Load the reagents and consumables based on the layout below. Press / Click *"CONTINUE"* when done. Refer to Appendix to view layout with 3 and 4 deepwell plates.

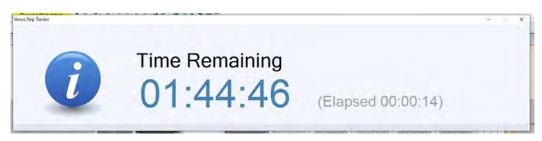
00uL lips			DWP1	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	Lysis
00uL Tips									Beads
uffer D2		T							000000
Buffer D3									
	Buffer D4					1000uL Tips	50uL Tips		

1.2.6. Scan the ViroKey[®] HT Total Nucleic Acid Kit label. Press / Click *"CONTINUE"* when done.

For Prescription Use Only



1.2.7. Wait for extraction to be completed. Extraction takes approximately 1 hour 45 minutes to complete.



1.2.8. Press / Click *"Unload All Carriers"* after the run is completed. Unload all carriers, clean and disinfect the Hamilton Microlab[®] STAR[™] instrument after each run. For instrument maintenance, please refer to the Hamilton Microlab[®] STAR[™] instrument maintenance videos.

For Prescription Use Only



Proceed to "3. HT PCR Setup", on the "Hamilton Method Manager" user interface, after the run is completed.

1.3. HT PCR Setup

Figure 3 shows the positions of consumables / labware on the Hamilton Microlab[®] STARTM platform. Double line the *w*aste bin with biohazard bags. Please refer to the layout as indicated by the Hamilton Microlab[®] STARTM instrument software or the appendix to load all items in the correct positions.

•	DWP1	PCR Plate 1	50uL Tips		K - J	
				103		
				1000uL Tips		
		1 PCR Strip	300uL Tips			PCR O MM

Figure 3. Layout of the Hamilton Microlab[®] STAR[™] platform for 1 sample plate for HT PCR setup (refer to Appendix for 2 to 4 sample plate layouts).

NOTE:

- Items shown are necessary for PCR assay setup for application "STAR8AL96 Vela_PCRsetup_V1.3.med".
- Ensure all consumables / labware are properly placed, aligned and secured into their respective positions.
- Ensure that the biohazard bags are properly attached to the waste bin before starting a protocol run. For more information, please refer to the Hamilton Microlab[®] STARTM instrument user manual.

For Prescription Use Only

1.3.1. Press / Click the "RUN" under "3. HT PCR Setup".

	1. Sample Plate				
		-			close [x]
	Preparation	Run	Layout	Summary	Anex
	2. Viral RNA Extraction	b Run	Layout	Summary	Anex
VIROKEY	3. HT PCR Setup	Pun Run	Layout	Summary	Anex
Frequent Used Methods:					
2. Viral RNA Extraction	3. HT PCR Setup	1. Sam	ole Plate Preparation	Independen Tra	nt Channel Tip

1.3.2. Input value 1 to 4 (minimum 1; maximum 4) for the *"Number of Sample Plate"*.

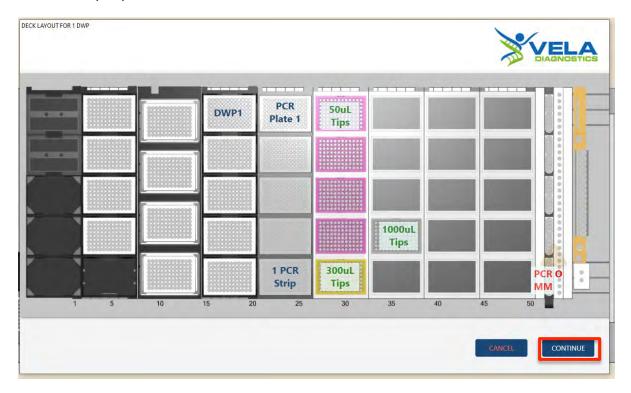


Press / Click "CONTINUE" when done.

For Prescription Use Only

1.3.3. Load the reagents and consumables based on the layout below. Press / Click

"CONTINUE" when done. Refer to Appendix to view layout with 2 to 4 sample plates.



NOTE: PCR Strip in the layout refers to HT 8-Strip Tubes.

For Prescription Use Only

1.3.4. Scan the PCR Kit 1 (HT M1 of ViroKey[®] HT SARS-CoV-2 RT-PCR Test kit)

label. Press / Click "CONTINUE" when done.

Scan Barcode:	
Scan Barcoue.	
M1 Barcode:	
нт ма	
Bill Lancing Surf	
	M1 Barcode:

1.3.5. Scan the PCR Plate 1 label. Place plate on PCR plate carrier, Track 21 – 26, Position 1. Press / Click *"CONTINUE"* when done.



1.3.6. [If applicable] Repeat step 1.3.4 to step Error! Reference source not found. and for PCR Plates 2 to 4 if performing for 2x96 to 4x96 tests respectively.

NOTE:

Track	PCR Plate	Position
	1	1
21 – 26	2	2
21 - 20	3	3
	4	4

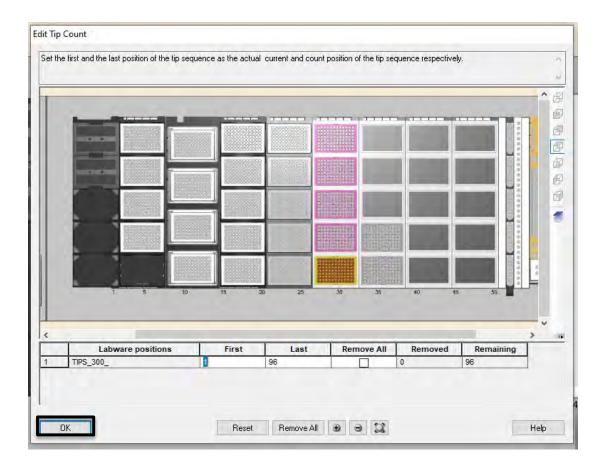
1.3.7. Input the number of 300 μL tips used under the *"First"* and *"Last"* columns or select the area where the *"First"* and *"Last"* samples are placed using the cursor (by *"clicking"*, *"holding"* and *"dragging"* over an area). Press / Click *"OK"*

when done.

NOTE:

- After the user has indicated the *"First"* and *"Last"* samples, location of the samples are shown as "brown circles".
- Remove the leftover tips (if any) and use them for the next PCR setup.

For Prescription Use Only

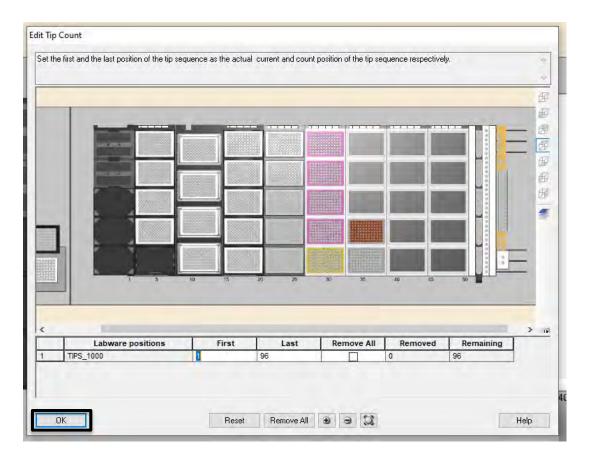


1.3.8. Input the number of 1,000 μL tips used under the *"First"* and *"Last"* columns or select the area where the *"First"* and *"Last"* samples are placed using the cursor (by *"clicking"* and *"dragging"* over an area). Press / Click *"OK"* when done.

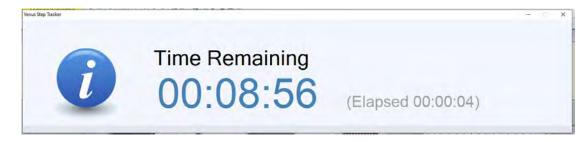
NOTE:

- After the user has indicated the *"First"* and *"Last"* samples, location of the samples are shown as "brown circles".
- Remove the leftover tips (if any) and use them for the next PCR setup.

For Prescription Use Only



1.3.9. Wait for PCR setup to be completed. PCR setup takes approximately 9 minutes to complete.



For Prescription Use Only

1.3.10. Press / Click *"Unload All Carriers"* after the run is completed. Unload all carriers, clean and disinfect the Hamilton Microlab[®] STAR[™] instrument after each run. For instrument maintenance, please refer to the Hamilton Microlab[®] STAR[™] instrument maintenance videos.



After the run is completed, carefully remove the MicroAmp[®] Fast Optical 96-Well Reaction Plate. Apply the MicroAmp[®] Optical Adhesive Film over the plate and seal the plate tightly to prevent contamination. Briefly spin down the PCR plate and load it onto the *Sentosa[®]* SA201 Real-Time PCR Instrument. Proceed to RT-PCR and data analysis using the *Sentosa[®]* SA201 Real-Time PCR Instrument and *Sentosa[®]* SA201 Real-Time PCR Instrument and *Sentosa[®]* SA201 Reporter (page 47 to page 63).

After RT-PCR and data analysis are complete, proceed to page 64 for result interpretation after data analysis.

PCR and data analysis using the Sentosa[®] SA201 Real-Time PCR Instrument and Sentosa[®] SA201 Reporter software

2. PCR on the Sentosa[®] SA201 Real-Time PCR Instrument

2.1. Switch on the Sentosa[®] SA201 Real-Time PCR Instrument by pressing the power button on the instrument.
 NOTE: Ensure the green indicator is lit and not flashing.

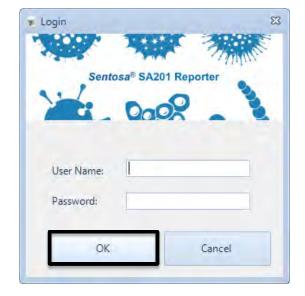


Virokey III JAND-COV-2 III-I CR Test Instructions for Use

2.2. Launch the *Sentosa*[®] SA201 Reporter software by double-clicking on the icon.



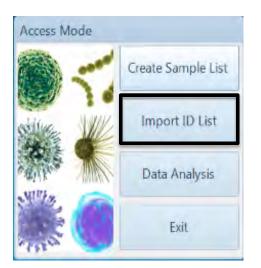
Type the user name and password, and then click "OK".



2.3. After logging in, "Access Mode" pop-up dialog is displayed.

Click *"Import ID List"* under *"Access Mode"* pop-up dialog to import the required *".smp"* file generated by the Hamilton Microlab[®] STARTM instrument.

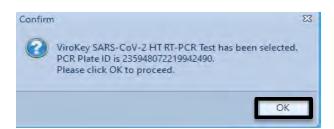
For Prescription Use Only



Home Share	View				21.0	1 million and the second	
* This	PC > Windows (C:) > VelaDx	*			~ 0	JD Search VelaDx	
Quick access	Name	Date modified	Type	Size			
Desktop *	PCR_210521 1434_bmngvh.smp	21/5/2021 2:38 pm	SMP File	58 KB			
	PCR_210521 1434_bmngvh.xml	21/5/2021 2:38 pm	XML Document	57 KB			
Downloads *	PCR_210521 1434_nfgngfc.xml	21/5/2021 2:38 pm	XML Document	O KB			
Documents 💉	PCR_210521 1434_bnfng.smp	21/5/2021 2:38 pm	SMP File	58 KB			
Pictures #	PCR_210521 1434_bnfng.xml	21/5/2021 2:38 pm	XMI Document	57 KB			
HAMILTON *	PCR_210521 1434_gbfgvcb.smp	21/5/2021 2:38 pm	SMP File	58 KB			
Methods at	PCR_210521 1434_gbfgvcb.xml	21/5/2021 2:38 pm	XML Document	57 KB			
LogFiles #	PCR_210521 1429_123nfgfg.smp	21/5/2021 2:33 pm	SMP File	58 KB			
VelaDx #	PCR_210521 1429_123nfgfg.xml	21/5/2021 2:33 pm	XMI Document	57 KB			
and the second second second	PCR_210519 1734_12345.xml	19/5/2021 5:36 pm	XML Document	O KB			
Hamilton screenshc	PCR_210506 1403_CF9244H6.smp	6/5/2021 2:15 pm	SMP File	57 KB			
Kit Pictures	PCR_210506 1403_CF9244H6.xml	6/5/2021 2:18 pm	XML Document	57 KB			
Methods	PCR_210506 1405_CF9244H5.smp	6/5/2021 2:18 pm	SMP File	55.8.8			
Virokey	PCR_210506 1403_CF9244H5.xml	6/5/2021 2:18 pm	XML Document	58 KB			
OneDrive	PCR_210429 1435_CF924407.smp	29/4/2021 2:41 pm	SMP File	57 KB			
Uneprive	PCR_210429 1435_CF9244J7.xml	29/4/2021 2:41 pm	XML Ducument	57 KB			
This PC	PCR_210429 1435_CF924406.smp	29/4/2021 2:40 pm	SMP File	57 KB			
ALC: NO	C PCR_210429 1435_CF9244J6.xml	29/4/3021.2540 pm	XMI Document	57 KB			
Network.	PCR_210429 1435_CF9244J5.smp	29/4/2021 2:40 pm	SMP File	57 KB			
	PCR_210429 1435_CF9244J5.xml	29/4/2021 £540 pm	XML Document	57 KB			
	PCR_210429 1435_CF924404.smp	29/4/2021 2:40 pm	SMP File	57 KB			
	PCR_210429 1435_CF9244I4.xml	29/4/2021 2:40 pm	XML Document	57 KB			
	PCR_210429 1332_CF9244I0.smp	29/4/2021 1:45 pm	SMP File	57 KB			
	PCR_210429 1332_CF9244I0.xml	29/4/2021 1-45 pm	SML Document	57 KB			
	PCR_210429 1332_CF924411.smp	29/4/2021 1:45 pm	SMP File	57 KB			
	PCR_210429 1332_CF9244I1.xml	29/4/3021 1:45 pm	XMI Document	57 KB			
	PCR_210429 1332_CF9244I2.smp	29/4/2021 1:44 pm	SMP File	57 KB			
	PCR_210429 1332_CF9244I2.xml	29/4/2021 1:44 pm	XML Document	57 KB			
	PCR_210429 1332_CF9244I3.smp	29/4/2021 1s44 pm	SMP File	57 KB			
	PCR_210429 1332_CF9244I3.xml	29/4/2021 1:44 pm	XML Document	57 KB			
	PCR_210429 1010_11111.smp	29/4/2021 10:12 am	SMP File	58 KB			
	PCR_210429 1010_11111.xml	29/4/2021 10:12 am	XVVL Document	58 KB			
	PCR_210429 1006_22222.smp	29/4/2021 10:08 am	SMP File	58 KB			
	PCR_210429 1006_22222.xml	29/4/2021 10:08 am	XMI Document	58 KB			
	PCR 210429 1005 33333.smp	29/4/2021 10:08 am	SMP File	58 KB			

2.4. After the ".*smp*" file has been imported, the software will require a confirmation on the selected assay. Click "OK" to confirm the sample layout and the information displayed from the ".*smp*" file.

For Prescription Use Only



	Samples	Run	Analys	is 🔪	Report
Fest Name: /iroKey SARS-CoV-2 HTRT-PCR Test	Display Test Target:	All tests/ targets	🔹 🔲 Well Sorting		
Operator:	Test/Target	Well	Sample Name	Sample Type	M1
operator	SARS-CoV-2-HT	A1	V*202258*NC**1234567	NC	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	B1	V*202259*PC**2345678	PC	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	C1	SMP0023	Sample	V*202255*M1*15*WT12345
emplate:	SARS-CoV-2-HT	D1	SMP0035	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	El	SMP0047	Sample	V*202255*M1*15*WT12345
roKey SARS-CoV-2 TRT-PCR Test.sdt	SARS-CoV-2-HT	F1	SMP0059	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	G1	SMP0071	Sample	V*202255*M1*15*WT12345
otes:	SARS-CoV-2-HT	H1	SMP0083	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	A2	SMP0001	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	B2	SMP0012	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	C2	SMP0024	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	D2	SMP0036	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	E2	SMP0048	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	F2	SMP0060	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	G2	SMP0072	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	H2	SMP0084	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	A3	SMP0002	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	B3	SMP0013	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	C3	SMP0025	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	D3	SMP0037	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	E3	SMP0049	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	F3	SMP0061	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	G3	SMP0073	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	H3	SMP0085	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	A4	SMP0003	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	B4	SMP0014	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	C4	SMP0026	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	D4	SMP0038	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	E4	SMP0050	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	F4	SMP0062	Sample	V*202255*M1*15*WT12345
	SARS-CoV-2-HT	G4	SMP0074	Sample	V*202255*M1*15*WT12345
					7
out Contact Us	Help				Next >>



2.5. Activate *"Insert the plate and close the tray"* checkbox to activate *"Step 2: Launch Sentosa[®] SA201 Series Software"*. Click on *"Start SA201"* button.

For Prescription Use Only

Sentosa SA201 Reporter					
	Samples	Run	Analysis	Report	<u>}</u>
Test Name: ViroKey SARS-CoV-2 HT RT-PCR Test. Operator:	Step 1: Insert the plate and o Run ID: ViroKey HT SARS-Co		Step	92: Launch Sentosa® SA201 Series S	oftware
emplate: //roKey SARS-CoV-2 4T RT-PCR Testsadt Notes:					
		_			
	4		•	A	
				St	art SA201
out 🕴 <u>Contact Us</u> 🕴 <u>H</u>	telp << Previous			/ [.	Next >>

2.6. A "Confirm" dialog box will be displayed. Click "OK".

For Prescription Use Only

entosa SA201 Reporter					- 0
	Samples	Run	Analysis	Report	
Test Name: VroKey SARS-CoV-2 IT RT-PCR Test	Step 1: Insert the p	alate and close the tray	S	tep 2: Launch Sentosa® SA201 Series S	Software
perator:	Run ID: ViroKey I F	T SARS-CoV-2 RT-PCR Test_12			
mplate:	2	-			
roKey SARS-CoV-2 FRT-PCR Test.sdt		Confirm	🛛 ncelled once started. Please confirm.		
- 1	4-		OK Cancel		
•				St	art SA201
out 🕴 Contact Us 🕴 <u>H</u>	lelp << Previous				Next >>

2.7. Another "Confirm" dialog box will be displayed. Click "OK".



2.8. The *Sentosa*[®] SA201 Reporter will begin the process (automatically) of logging in to the *Sentosa*[®] SA201 Series Software and running a series of steps on it to start

For Prescription Use Only

the new run. A pop-up window titled *"Running in Progress"* will be displayed throughout this process, reminding the user not to perform any other actions. The pop-up window will also indicate the individual specific steps that are being performed in the background.

	Samples F	Run	Analysis	Report
est Name: iroKey SARS-CoV-2 T RT-PCR Test	Step 1: Insert the plate and close the tray Run ID: ViroKey HT SARS-CoV-2 RT-PCR		Step 2: Launc	n Sentosa® SA201 Series Software
perator:				
mplate:				
roKey SARS-CoV-2 T RT-PCR Test adt				
Notes:		9.5		
	Running in Progress			
	Logging in to the instru Please do not perform	ument	until this non-un mos	sago disappoars
	Please do not perform	any other actions	unui uns pop-up mes	saye usappears.
-				
				Start SA201
out Contact Us				Start SA201

The following is the list of automated steps that will be performed on the *Sentosa*[®] SA201 Series Software:

- I. Logging in to the *Sentosa*[®] SA201 Series Software using the same user account credentials that were used to log in to the *Sentosa*[®] SA201 Reporter.
- II. Importing the sample setup file which was generated by the *Sentosa*[®] SA201 Reporter.
- III. Saving the run document (".sds") file.
- IV. Starting the run.

Please do not perform any other action during the automated process. The run will be started on the *Sentosa*[®] SA201 instrument at the end of the process. This can be verified by selecting the *"Instrument"* tab and checking the *"Instrument Control"* panel on the *Sentosa*[®] SA201 Series Software.

For Prescription Use Only

Image: Stature to the substanded Time Remaining (Mr.mm): Temperature Stature to the substanded Time Remaining (Mr.mm): Temperature to the substanded Time Remaining (Mr.mm): Stature to the substanded Time Remaining (Mr.mm): Temperature to the substanded Time Remaining (Mr.mm): Stature to the substanded Time Remaining (Mr.mm): Temperature to the substanded Time Remaining (Mr.mm): Stature to the substanded Time Remaining (Mr.mm): Temperature to the substanded Time Remaining (Mr.mm): Stature to the substanded Time Remaining (Mr.mm): Stature to the substanded Time Remaining (Mr.mm): Stature to the substanded Time Remaining (Mr.mm): Stature to the substanded Time Remaining (Mr.mm): Stature to the substanded Time Remaining (Mr.mm): Stature to the substanded Time Remaining (Mr.mm): Stature to the substanded Time Remaining (Mr.mm): Stature to the substanded Time Remaining (Mr.mm): Stature to the substanded Time Remaining (Mr.mm): Stature to the substanded Time Remaining (Mr.mm): Stature to the substanded Time Remaining (Mr.mm): Stature to the substanded Time	File View Tools 21CFR11 Instrument Analysis	Window Help	- B
up // Instrument VResults // Audit Trail / E-Signatures strument Control Strument Control <t< th=""><th>🖻 🖃 🎒 🖪 國 🗹 🖬 🕨 💽 🤗</th><th></th><th></th></t<>	🖻 🖃 🎒 🖪 國 🗹 🖬 🕨 💽 🤗		
Statu Status: Extend. Stage 1 Stage 2 Stage 1 Stage 2 Stage 3 Rept: Thermal Prolife Auto Increment Rept: Stage 1 Stage 2 Stage 3 Rept: Stage 1 Stage 1 Stage 2 Stage 3 Rept: 1 3:00 0:15 5:00 0:26 Help Sample Volume (µL): 25 Run Mode Fast 7500 Expert Mode Solid Crole		es	
Statu Status: Status: <	trument Control	Temperature	
Stop Cover: Block: Cycle Stage: Rep: Time (mmss): Step: Stage 1 Stage 2 Reps Stage 3 Reps Reps 95.0 95.0 95.	Estimated Time Remaining (hh:mm):		
Discomment Status: Cycle Repr. Extend. Time (minss): Step: Extend. State: State:		Cover: Block:	
Extend Time (mm:ss): Step: stage 1 Stage 2 Stage 3 Reps: 1 Reps: 1 Reps: 1 0 0:15 0:0 0 0:15 0:0 0 0:15 0:0 0 0:15 0:0 0 0:15 0:0 0 0:15 0:0 0 0:0 0:0 15:00 0:0 0:0 4dd Cycle Add Hold Add Step Delec Settings Sample Volume (µL): 26 Run Mode Fast 7500 Expert Mode Select /Weav Hilfers.		Cycle	
Extend State: nermal Protie Auto Increment Ramp Rate Stage 1 Stage 2 Stage 3 Reps: Reps: Reps: Reps: 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Jisconnect Status:		
remal Cycler Protocol Thermal Profile Auto Increment Ramp Rate Stage 1 Stage 2 Stage 3 Reps: T Reps: 1 Reps: 40 95.0 95.0 95.0 95.0 0:15 50.0 0:16 0:30 15:00 0:16 0:30 Add Cycle Add Hold Add Step 0:elete Help Settings Sample Volume (µL): 26 Run Mode Fast 7500 F Expert Mode Select // Waw Filters.	Entrant		
Themal Profile Auto Increment Ramp Rate Stage 1 Reps: T Reps: T Reps: T Reps: T Reps: 1 Reps:	Dxtend.	State:	
Themal Profile Auto Increment Ramp Rate Stage 1 Reps: T Reps: T Reps: T Reps: T Reps: 1 Reps:	ermal Cycler Protocol		
Stage 1 Stage 2 Stage 3 Reps: 1 Reps: 80 95.0 95.0			
Reps: Reps: Reps: 40 95.0 95.0 95.0 95.0 <td< td=""><td></td><td></td><td></td></td<>			
300 0:16 50.0 0:30 15:00 0:30 Add Dycle Add Hold Add Cycle Add Hold Add Cycle Add Step Detete Help Settings Sample Volume (µL): 26 Run Mode Fast 7500 T	Reps: 1 Reps: 1 Reps: 40		
300 0:16 50.0 0:30 15:00 0:30 Add Dycle Add Hold Add Cycle Add Hold Add Cycle Add Step Detete Help Settings Sample Volume (µL): 26 Run Mode Fast 7500 T	95.0 95.0		
50.0 58.0 115.00 0:30 Add Cycle Add Hold Add Step Delete Help Settings Sample Volume (µL): 26 Run Mode Fast 7500 T Expert Mode Select /\view Filters.			
Add Cycle Add Hold Add Step Delete Help Settings Sample Volume (µL): 26 Run Mode Fast 7500 Expert Mode Select /Wew Filtensy,	3:00 0:15		
Add Cycle Add Hold Add Step Dieletc Help Settings Sample Volume (µL): 26 Run Mode Fast 7500 If Expert Mode Select /Viaw Filters	58.	0	
Add Cycle Add Hold Add Step Delete Settings Sample Volume (µL): 26 Run Mode Fast 7500 T Expert Mode Select ///www Filters.	0:3	0	
Settings Sample Volume (µL): 26 Run Mode Fast 7500 ✓ F Expert Mode Select /Viaw Filters.	15:00		
Settings Sample Volume (µL): 26 Run Mode Fast 7500 ✓ F Expert Mode Select /Viaw Filters.			
Settings Sample Volume (µL) : 26 Run Mode Fast 7500 ▼ Fixpert Mode Select /View Filters.			
Settings Sample Volume (μL): 26 Run Mode Fast 7500 T Expert Mode Select /Wew Filters.	and the second second second	i i i i i i i i i i i i i i i i i i i	
Sample Volume (µL): 26 Run Mode Fast 7500 T Expert Mode Select / View Filtres.	Add Cycle Add Hold Add Step Delete	Help	
Run Mode Fast 7500 T Expert Mode Select / View Filtres.	Settings		
	Sample Volume (µL): 26		
	Bun Mode Fast 7500	Expert Mode Select/View Filters.	
Data Collection : Stage 3, Step 2 (58.0 @ 0.30)			
	Data Collection : Stage 3, Step 2 (58.0 @ 0:30)		

2.9. [Optional] If the automated process is stopped or interrupted due to any unforeseen circumstances, the run can be resumed manually by following the

For Prescription Use Only

steps listed below. Please skip any steps that have already been completed by the automated process before the interruption occurs.

2.9.1. Log in to the *Sentosa*[®] SA201 Series Software by typing the user name and password in the *"User Login"* pop-up window. Click *"OK"*.

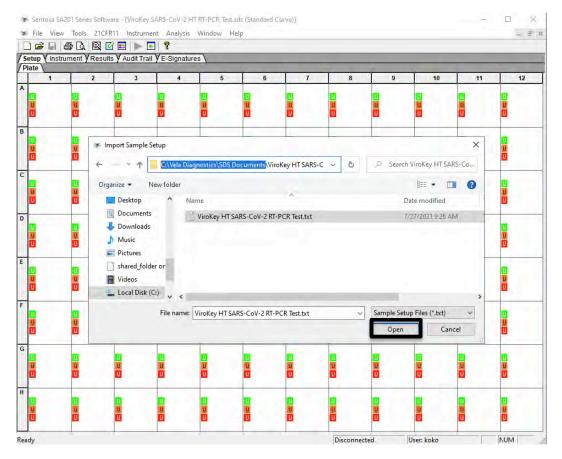
entosa SA201 Reporter	Samples	Run	Analy	sis	Report	
Fest Name: irroKey SARS-CoV-2 IT RT-PCR Test. Operator:		plate and close the tray PSARS-CoV-2 RT-PCR Test_03		Step 2: Launch Si	entosa® SA201 Series S	oftware
emplate:		User Login		×		
lotes:	4	Sentos	a [®] SA201 Series So	oftware		
		*	User name : Password : OK Cancel		Faces and the second	
2					St	art SA201
out 🕴 Contact Us 🎽 H	leip. << Previous	3				Next >>

2.9.2. Select *"File"* from the main menu and *"Import Sample Setup"* from the dropdown list. *"Import Sample Setup"* pop-up window will be displayed. The sample file is given a default file name *("Sentosa SA201 Assay name*

For Prescription Use Only

PCR/RT-PCR Test YYYY-MM-DD_HH-MM-SS.txt"). Select the *".txt"* file and click *"Open"*.

NOTE: DO NOT modify the generated *".txt"* file as this will result in an error.



The sample layout will change according to the imported sample setup file.

Sentosa SA201 Series Software - [ViroKey HT SARS-CoV-2 RT-PCR Test_22-07_2201.sds (Standard Curve)] \times 😸 File View Tools 21CFR11 Instrument Analysis Window Help _ 8 × 🗋 🚅 🗐 🎒 🖪 🖳 🖾 🖬 🕨 💽 📍 / Setup V Instrument V Results V Audit Trail V E-Signatures Plate \ 5 6 7 8 1 2 3 4 9 10 11 12 A V*202258*NC* SMP0001 SMP0002 SMP0003 SMP0004 SMP0005 SMP0006 SMP0007 SMP0008 SMP0009 SMP0010 SMP0011 B V*202259*PC* SMP0012 SMP0013 SMP0014 SMP0015 SMP0016 SMP0017 SMP0018 SMP0019 SMP0020 SMP0021 SMP0022 С SMP0023 SMP0024 SMP0025 SMP0026 SMP0027 SMP0030 SMP0031 SMP0032 SMP0033 SMP0034 SMP0028 SMP0029 D SMP0035 SMP0036 SMP0037 SMP0038 SMP0039 SMP0040 SMP0041 SMP0042 SMP0043 SMP0044 SMP0045 SMP0046 E SMP0047 SMP0048 SMP0049 SMP0050 SMP0051 SMP0052 SMP0053 SMP0054 SMP0055 SMP0056 SMP0057 SMP0058 F SMP0059 SMP0060 SMP0061 SMP0062 SMP0063 SMP0064 SMP0065 SMP0066 SMP0067 SMP0068 SMP0069 SMP0070 G SMP0071 SMP0072 SMP0073 SMP0074 SMP0075 SMP0076 SMP0077 SMP0078 SMP0079 SMP0080 SMP0081 SMP0082 H SMP0083 SMP0084 SMP0085 SMP0086 SMP0087 SMP0088 SMP0089 SMP0090 SMP0091 SMP0092 SMP0093 SMP0094 NUM Disconnected User: koko

For Prescription Use Only

2.9.3. Select *"File"* from the main menu and *"Save"* from the drop-down list. *"Reason for Change Entry"* window will be displayed. Describe the changes made in the document or simply enter *"Save file"* or any other suitable text in the textbox and click *"OK"* to save the run document (*".sds"*) file. **NOTE: DO NOT** modify the generated *".sds"* file as this will result in an error.

Pessent for Change Entry
Username: Velso:
Describe the changer that must been receipt in the Desument:
Save

2.9.4. Select the "Instrument" tab and click "Start" to start the run.

For Prescription Use Only

	Window Help	- B
🖻 🖬 🖨 🖪 國 🗹 🖬 🕨 💽 🤶		
tup YInstrument VResults Y Audit Trail YE-Signature	es	
istrument Control	Temperature	
Start Estimated Time Remaining (hh:mm):	Sample: Heat Sink: Cover: Block:	
Disconnect Status:	Cycle Stage: Rep:	
Extend.	Time (mm:ss): Step: State:	
hermal Cycler Protocol		
Thermal Profile Auto Increment Ramp Rate Stage 1 Stage 2 Stage 3		
Reps: 1 Reps: 1 Reps: 40		
95.0 95.0 3:00 0:15		
158.0		
50.0		
Y		
Add Cycle Add Hold Add Step Delete	Help	
Settings	Help	
Settings Sample Volume (µL) : 26		
Settings Sample Volume (µL) : 26 Run Mode Fast 7500	Expert Mode Select/View Hiters.	
Settings Sample Volume (µL) : 26	Expert Mode Select/View Filters.	
Settings Sample Volume (µL) : 26 Run Mode Fast 7500	Expert Mode Select/View Hiters.	
Settings Sample Volume (µL) : 26 Run Mode Fast 7500	Expert Mode Select/View Hiters.	
Settings Sample Volume (µL) : 26 Run Mode Fast 7500	Expert Mode Select/View Hiters.	
Settings Sample Volume (µL) : 26 Run Mode Fast 7500	Expert Mode Select/View Hitters.	
Settings Sample Volume (µL) : 26 Run Mode Fast 7500	Expert Mode Select/View Hitters.	

2.10. At the end of the run, a confirmation pop-up window will be displayed indicating that the run has completed. A window will prompt the user if result analysis on the *Sentosa*[®] SA201 Series Software is to be performed automatically or manually.



Click "Yes" to perform the result analysis automatically by using preconfigured analysis settings. A pop-up window titled "Analysis in Progress" will be displayed briefly, reminding the user not to perform any other actions.

The pop-up window will disappear when the result analysis has completed successfully. The *Sentosa*[®] SA201 Series Software may now be closed by clicking the close button " and the title bar. On the *Sentosa*[®] SA201 Reporter, click "*Next*" to proceed to data analysis.

2.10.1 [Optional] In the previous step, click "No" if the user wishes to make changes to preconfigured analysis setting(s) before performing the result

For Prescription Use Only

analysis. Click "OK" on the pop-up window with the message "The run completed successfully". Log in to the Sentosa[®] SA201 Series Software if "Idle Timeout re-authentication required" pop-up window is prompted.

On the *Sentosa*[®] SA201 Series Software, change the analysis setting(s) as necessary. Select *"Analysis"* from the main menu and *"Analyze"* from the drop-down list. *"Reason for Change Entry"* window will be displayed. Enter *"Analysis"* or any other suitable text in the text box and click *"OK"* to perform the result analysis.

Setup	Vinstrume	ent YResu	Its Y Aud	It Trail YE-Signat	ures		-		-	_	-
Plate \	-	100	-			1 1	-	-	and the second		
	1	2	3	4 5	5	7	8	9	10	11	12
			0	W20220541 S 1173 alysis Settings - Sta		3 5 1197 83 5	1200	1208 85			1.5
			1000	Ct Analysis Ct Analysis	ndard curve			~			
			- 11	C. Andros							
				Detector: AI			-			1	
			- 11	Sector 1							
		1.1		C Auto Ct							
				Manual Ct							
-			- 11	Threshold: [ma	(ed)				_		
	1.1	1.1		C. C. T. S.					641		
				C Automatic Ba	aseline	1. Carlos 1.					
			-11	Manual Base	aine Start (cyc	le) ⁵ Er	nd (cycle)	2			
								1 A A			
	- i- i-			T Use System Cá	llusio				_		
		111	1	1 00000000000000	- Constant						
	4.14			OK & Reanalyze	OK	Cancel	Арр	ly		1.1	
-	-			S 1171	87 5 4400 B	1 6 1100	1206 82 8	RECORDENSE			
					U		1240-04	a da			
								Contraction of			1111

The *Sentosa*[®] SA201 Series Software may now be closed by clicking the close button " I on the title bar. On the *Sentosa*[®] SA201 Reporter, click *"Next*" to proceed to data analysis.

For Prescription Use Only

3. Automated data analysis

 C_T values for each channel will be displayed under "Analysis" tab. Click "Next" to switch to "Report" tab to generate report.

VELA	Sar	mples		Ru	ŋ	Analysis	/	Report				
lest Names Hokey SAKS-Cell-a Hi KI-PCK Test	Start:	5	End:	12		Threshold: 0.02						Delta Rn
	Well	Sample ID	Туре	Ct	Pag ^	GREEN (SARS-CoV-2-HT) (RANGE (SARS-	CoV-2-HT) R	ED (SARS-CoV-2-H	T)		
Operator:	V A1		NC		SAF			GDEEN (SADS	-CoV-2-HT) Rn vs Cvc			
Lease of the second	I B1	V*202259*PC**T	XI PC	23.68	SAF			UNCEN (OANS	-cov-z-iii) iurvs cyc	•		
	🔽 C1	1986HK_750_Ref	Sample	32.48	SAF	4.000e+006						1
	🔽 D1	NC_Ref	Sample		SAF							11
emplate	📝 E1	HTPC_Ref	Sample	23.81	SAF							
Vienka, thorney :		Sample	31.45	SAF	3.000e+006 -						11/1	
HT RT DCB Testadt	📝 G1	NC_Ref	Sample	le SAF	0.0000 000						1111	
	📝 H1	HTPC_Ref	Sample	23.56	SAF							
Notes:	V A2	1986HK_750_Ref	Sample	31.36	SAF							
lotes	I B2	NC_Ref	Sample		SAF	튠 2.000e+006 -						
	V C2	HTPC_Ref	Sample	23.78	SAF							
	🔽 D2	1986HK_750_Ref	Sample	30.95	SAF							
	₩ E2	NC_Ref	Sample		SAF							
	V F2	HTPC_Ref	Sample	23.51	SAF	1.000e+006 -					//	
	🔽 G2	1986HK_750_Ref	Sample	31.84	SAF						1	
	✓ H2	NC_Ref	Sample		SAF		_	_				-
	📝 A3	HTPC_Ref	Sample	23.94	SAF	0.000e+000						
	📝 B3	1986HK_750_Ref	Sample	31.09	SAF	Ó	5	10 1		25	30	35 40
	🗹 C3	NC_Ref	Sample		SAF ↓				Cycle Number			
	<				>							
	Check	All Uncheck	All				Linear	Scale Auto	Scale Zoom Ir	Zoom O	ut	

Under "Analysis" tab, there are other functions available as described below:

- Delta Rn is the magnitude of the fluorescence signal generated during the PCR at each time point. Delta Rn curve represents the signal processed from the raw data.
- Click *"Linear Scale"* to display the Y-axis in linear scale.
- Click "Log Scale" to display the Y-axis in log scale.
- Click "Check All" / "Uncheck All" to select / unselect all samples from the list. Only the selected sample curves will be displayed.
- To enlarge a particular area of a sample curve, click "Zoom In" and select the area of interest. Click "Zoom Out" to decrease the magnification of the curve.
- Click "Auto-Scale" to display all selected sample curves automatically to a standard size.

3.1. Report generation

Under "*Report*" tab, there are other functions available as mentioned below:

- Activate "Filter" checkbox to display the selected samples, or deactivate "Filter" checkbox to hide the selected samples. User can also select individual samples by activating the checkbox for each sample. Only selected samples will be displayed in the report.
- Click "III" icon to group the samples with same sample ID. If user selects one sample ID, all the grouped samples will be selected together.
- Activate the checkboxes under *"Experimental Information"* to allow the user to customize the report.
 - Activate "Quantitation Information" checkbox to display assay analysis settings for each fluorescence channel.
 - Activate "Cycling Profile" checkbox to display the run parameter settings.
 - Activate "Raw / Normalized Curve" checkbox to display both raw and normalized curves for each fluorescence channel.
 - Activate "Message" checkbox to display any pre-defined message for the run.
- *"Result"* column will display the test result of the samples for the run.
- *"Validity"* column will display the validity of the sample for the run.
- *"Fluorescence channels C_t"* column will display the C_t value for the samples.

Test Names	E Filter	Target :	All Targets		Well Sorting					Preview Report
n okey saks-celle Hi ki PCR rol	Color	Wel	▲ Sample	SampleType	Test	M1-1 GREEN (Ct)	M1-1 RED (CI)	M1-2 GREEN (CI)	M1-2*	Experimental Information
Operator:	×.	82 88	V*202198*PC**1000000581	PC	SARS-CoV-2	27.62	26.28	34.18	26.47	Quantitation Information
operation	(2)	C2 C8	PC_M1-1	Sample	SARS-CoV-2	27.13	26.38	33.79	26.08	Raw/ Normalized Curve
	12	D2 D8	PC_M1-1	Sample	SARS-CoV-2	27.62	26.59	37.70	26.63	✓ Message
Template	12	83 89	10_M1-1	Sample	SARS-CoV-2	30.64	27.03	35.51	26,44	
V rolley SARS-CeV-1 HT RT-PCR Test.idt	V.	C3 (9	10_M1-1	Sample	SARS-CoV-2	31.50	26.90	34.11	26.10	
Notes	2	D3 D9	10_M1-1	Sample	SARS-CoV-2	31.56	26.69	36.48	26.32	
	120	E3 E9	10_M1-1	Sample	SARS-CoV-2	32.10	26.60	44.83	26.48	
	10	F3 F9	10_M1-1	Sample	SARS-CoV-2	30.90	26.89	35.53	26.27	
	12	H3 H9	V*202205*NC**1000000580	NC	SARS-CoV-2		25.97		25,74	
	191	84 810	10_M1-1	Sample	SARS-CoV-2	32.06	26.68	36,44	26,47	
	2	C4 C10	10_M1-1	Sample	SARS-CoV-2	30.27	27.74	34.16	26.14	
	12)	D4 D10	10_M1-1	Sample	SARS-CoV-2	31.30	26.74	41.63	26.22	
	101 4	E4	10 MI.1	Camala	CARC.CoV.7	27.40	76 00	41 62	76 17 ×	

For Prescription Use Only

Viroley BARCodes Color Weiley Sample Virokey HT SARS-CoV-2 RT-PCR Test_20210416_HT_ReagentStability_Run01 Experiment Operator: 0 A1 Vr202259 Experiment Information ato PC; Experiment dio Samp; C Quantitation	Sentosa SA201 Reporter				* Report Preview			×	-
Test Nome File Target: All Target Corrant Color Well Sample Color Well Sample Color Well Sample Corrant Color Well Sample Color Well Sample Correct Color Well Sample Color Color Color Color Well Sample Color Color Color Color Color Color Well Sample Color Color Color Color Color Well Sample Color Color Color Color Color Color Color Color Color Color Color <t< th=""><th></th><th>San</th><th>nples</th><th>1</th><th></th><th></th><th>nd Next</th><th>~</th><th></th></t<>		San	nples	1			nd Next	~	
Hit Name Virokey HT SARS-CoV-2 RT-PCR Test_20210416_HT_ReagentStability_Run01 A Experiment Coperator 8 it Vr202259 • Experiment Information 0 to Sample 0 countitation Coperator 9 it 8 it Vr202259 • Experiment Information 0 to Sample 0 countitation Template: 0 to NC_Ref Rin ID Virokey HT SARS-CoV-2 RT-PCR 0 countitation		Filter	Target :	All Targets	Sentosa [®] SA201	Report	Y		Preview Report
Operator: Image: Construction Image: Construction </td <td></td> <td>Color</td> <td>Well</td> <td>Sample</td> <td>ViroKey HT SARS-CoV-2 RT-PC</td> <td>R Test_20210416_HT_ReagentStability_Run01</td> <td>-</td> <td>~</td> <td>Experimental Information</td>		Color	Well	Sample	ViroKey HT SARS-CoV-2 RT-PC	R Test_20210416_HT_ReagentStability_Run01	-	~	Experimental Information
Image: Section of the sectio		7	A1	V*202258*				d to PC.	Quantitation Information
Run ID Ymrokey IF SARS-CoV/2 RTP-CR Template Orl N.C.Ref Run ID Ymrokey IF SARS-CoV/2 RTP-CR Test 20210116 JF IF SARS-CoV/2 RTP-CR Test 20210116 JF IF SARS-CoV/2 RTP-CR Orl 2-viru	Operator:		B1	V*202259*	Experiment Inform	nation		d to Samp	Cycling Profile
Template: Dif Notest Fit 1986HtC7 Sentosa SA201 Series Software User: NSTR-ADMIN Sentosa SA201 Reporter User: NSTR-ADMIN Co-2-viru Workey MASCATE Fit 61 NC,Ref Run Signature ON OI-2-viru OI-	1	17	C1	1986HK 7				CoV-2 viru	Raw/ Normalized Curve
Template: Fil HTPC/R User Sentoss 3A201 Series Software User: INSTR-ADMIN CV-2 viru Virolay SACC Color Fil HTPC/R Notes: ON CV-2 viru CV-2 viru Notes: G1 NC,Ref Run Signature ON CV-2 viru CV-2 viru Notes: G2 HT HTPC/R Run Signature ON CV-2 viru CV-2 viru Notes: G2 NC,Ref Run Signature ON CV-2 viru CV-2 viru CV-2 viru Sentoss A201 Fepoter Version: 15.0003 Assay Package Version: 15.0003 CV-2 viru CV-2 viru CV-2 viru C2 HTPC/R Instrument Type Sentoss SA201 Fepoter Version: 15.0003 CV-2 viru CV-2 viru C2 NO. of SARS-CoV-2 HT M1 TX0004 CV-2 viru CV-2 viru CV-2 viru C2 NC.Ref Lot No. of SARS-CoV-2 HT M1 TX0004 CV-2 viru CV-2 viru C3 NC.Ref DList ViroKey HT SARS-CoV-2 RT-PCR Test CV-2 viru CV-2 viru C3 NC.Ref			D1	NC_Ref	Run ID			CoV-2 viru	Message
Template: FI 1980HK2 Sentosa SA201 Reporter User: INSTR.ADMIN OV-2 viru Volus VACCAGE G1 NC, Ref Run Signature ON OV-2 viru OV-2 viru Notes: H1 HTPC, Ref Run Signature ON Sentosa SA201 Reporter Vesion: V1 0.1 OV-2 viru OV-2 viru Notes: B2 NC, Ref Satta Modified 23-April-2021 16:26:23 OV-2 viru OV-2 viru OV-2 viru Sentosa SA201 Seises Software Version: V1 0.1 Sentosa SA201 Reporter Vesion: V1 0.1 OV-2 viru OV-2 viru OV-2 viru Sentosa SA201 Seises Software Version: 16.0017 RUO Instrument Type Sentosa SA201 Reporter Vesion: V1 0.1 OV-2 viru OV-2 viru C2 HTPC, Ref Instrument Type Sentosa SA201 Sentosa SA201 Reporter Vesion: V1 0.01 OV-2 viru C3 NC, Ref Instrument Type Sentosa SA201 ViroKey SARS-CoV-2 HT RT-PCR Test PCR OV-2 viru C3 NC, Ref ID List Name ViroKey HT SARS-CoV-2 RT-PCR Test OV-2 viru C3 NC, Ref ID List Name ViroKey HT SARS-CoV-2 RT-PCR Test OV-2			E1	HT PC_Re	11			CoV-2 viru	
Vinders DARSECACIÓN G1 NC,Ref Run Signature ON ON OV-2 viru OV-2 vir	Template		F1	1986HK_7	User			CoV-2 viru	
HT PC/CCText with HI HT PC/R Run Start 16-April-2021 17:54:58 CoV-2 viru CoV-2 viru <thc< td=""><td></td><td></td><td>G1</td><td>NC_Ref</td><td>Run Signature</td><td></td><td></td><td>oV-2 viru</td><td></td></thc<>			G1	NC_Ref	Run Signature			oV-2 viru	
Notes B2 NC,Ref B2 NC,Ref Domain Dowait Dowait <thdowait< th=""> Dowait Dowait</thdowait<>	HT RT PCR Test.idt		H1	HT PC_Re		16-April-2021 17:54:58		CoV-2 viru	
Notes: B2 NC_Ref Software Version Sentosa SA201 Series Software Version: v1.0.1 CoV-2 viru 2 C 2 HTPC_R8 Antone Sentosa SA201 Series Software Version: v1.0.1 CoV-2 viru CoV-2 viru 2 D 2 1998HC/1 Antone Sentosa SA201 Series Software Version: v1.0.1 CoV-2 viru CoV-2 viru 2 NC_Ref Instrument Type Sentosa SA201 Sentosa SA201 CoV-2 viru CoV-2 viru 2 10 Co 10 Sentosa SA201 CoV-2 viru CoV-2 viru 3 HTPC_R8 Sentosa SA201 Sentosa SA201 CoV-2 viru CoV-2 viru 2 10 NC_Ref Instrument Type Sentosa SA201 ViroKey SARS-CoV-2 HT RT-PCR Test CoV-2 viru CoV-2 viru 2 3 198HC7 ID List ViroKey HT SARS-CoV-2 RT-PCR Test CoV-2 viru 3 198HC7 ID List Name ViroKey HT SARS-CoV-2 RT-PCR Test CoV-2 viru CoV-2 viru 3 198HC7 ID Creation Date 4/220201 6.0 PM CoV-2 viru CoV-2 viru			A2	1986HK_7	Last Modified	23-April-2021 16:26:23		CoV-2 viru	
C2 HTPC_Re Sentesa SA201 Reporter Version: 1.5.003 Assay Package Version: 1.6.0017 RUO CoV-2 viru E2 NC_Ref Instrument Type Sentosa SA201 CoV-2 viru CoV-2 viru E2 NC_Ref Instrument Type Sentosa SA201 CoV-2 viru CoV-2 viru E2 NC_Ref Instrument Type Sentosa SA201 CoV-2 viru CoV-2 viru E2 NC_Ref Instrument Type Sentosa SA201 CoV-2 viru CoV-2 viru E2 NC_Ref Instrument Type Sentosa SA201 CoV-2 viru CoV-2 viru E2 NC_Ref Instrument Type Sentosa SA201 ViroKey SARS-CoV-2 HT RT-PCR Test PCR CoV-2 viru E3 NGRHK7 ID List ViroKey HT SARS-CoV-2 RT-PCR Test CoV-2 viru E3 1986HK7 ID List Name ViroKey HT SARS-CoV-2 RT-PCR Test CoV-2 viru E3 1986HK7 ID Creation Date 4/22/2021 K16 CoV-2 viru E3 1986HK7 ID Creation Date 4/22/2021 K16 CoV-2 viru E4 Lovis Kit V'301089*EXTRX*00000000012022	Notes:		B2	NC_Ref	Software Version			CoV-2 viru	
1 102 1980H.0.1 102 1980H.0.1 102 <			C2	HTPC_Re	Solitary Colorin	Sentosa SA201 Reporter Version: 1.5.0003		CoV-2 viru	
F2 HTPC,Re Instrument type Sentosa SA201 OV-2 viru G2 1996HC FCR Volume South Sentosa SA201 OV-2 viru A3 HTPC,Re Sentosa SA201 OV-2 viru OV-2 viru B3 1996HC ViroKey SARS-CoV-2 HT RT-PCR Test PCR Test OV-2 viru C3 NC,Ref ID List ViroKey HT SARS-CoV-2 RT-PCR Test OV-2 viru B3 1996HC ViroKey HT SARS-CoV-2 RT-PCR Test OV-2 viru C3 NC,Ref ID List Name ViroKey HT SARS-CoV-2 RT-PCR Test OV-2 viru B3 1996HC ID Creation Date 4/22/2021 6 0 PM OV-2 viru B3 1998HC ID Creation Date 4/22/2021 6 0 PM OV-2 viru B3 1998HC ID Creation Date 4/22/2021 6 0 PM OV-2 viru v F3 NC,Ref ID List Kit V'301089*EXTRX*000000001*2022-01-31 P			D2	1986HK_7		Assay Package Version: 1.6.0017 RUO		CoV-2 viru	
Image: Figure 1 Figure 1 Lot No. of SARS-CoV-2HT M1 TX0004 CV-2viru Image: Figure 1 G2 1986HC7 CR CR CV-2viru Image: Figure 1 A3 HTP C, Ref Sentosa SA201 ViroKey SARS-CoV-2 HT RT-PCR Test PCR CV-2viru Image: Figure 1 A3 HTP C, Ref Sentosa SA201 ViroKey SARS-CoV-2 HT RT-PCR Test PCR CV-2viru Image: Figure 1 B3 1986HC7 ID List ViroKey HT SARS-CoV-2 RT-PCR Test CV-2viru Image: Figure 1 ID List Name ViroKey HT SARS-CoV-2 RT-PCR Test CV-2viru CV-2viru Image: Figure 1 ID Creation Date 4/22/2021 6.0 PM CV-2viru CV-2viru Image: Figure 1 NC, Ref ID Creation Date 4/22/2021 6.0 PM CV-2viru Image: Figure 1 NC, Ref ID Creation Date CV-2viru CV-2viru Image: Image: Image: Figure 1 V1301089*EXTRX*000000001*2022-01-31 CV-2viru 2viru			E2	NC_Ref	Instrument Type	Sentosa SA201		CoV-2 viru	
G2 1996H(-7) PCR Volume 26uL CV-2 viru A3 HTPC_R Sentoa SA201 ViroKey SARS-CoV-2 HT RT-PCR Test PCR CV-2 viru B3 1986H(-7) V'301087*PCRRv'000000000*2022-01-25 CV-2 viru C3 NC_Ref ID List ViroKey HT SARS-CoV-2 RT-PCR Test CV-2 viru C3 NC_Ref ID List Name ViroKey HT SARS-CoV-2 RT-PCR Test CV-2 viru E3 1998H(-7) ID List Name ViroKey HT SARS-CoV-2 RT-PCR Test CV-2 viru E3 1998H(-7) ID Creation Date 4/22/2021 6.0 PM CV-2 viru E3 1998H(-7) ID Creation Date 4/22/2021 6.0 PM CV-2 viru E3 NC,Ref ID Creation Date V'301089*EXTRX*000000001*2022-01-31 CV-2 viru V			F2	HT PC_Re				CoV-2 viru	
H2 NC.Ref Assay Kit Sentosa SA201 ViroKey SARS-CoV-2 HT RT-PCR Test PCR CoV-2 viru B3 1989HK7 ViroSite ViroKey MT SARS-CoV-2 RT-PCR Test CoV-2 viru C3 NC_Ref ID List ViroKey HT SARS-CoV-2 RT-PCR Test CoV-2 viru D3 HT PC_Re ID List Name ViroKey HT SARS-CoV-2 RT-PCR Test CoV-2 viru F3 1986HK7 ID Creation Date 4/22/2021 f 60 PM CoV-2 viru F3 1986HK7 ID Creation Date 4/22/2021 f 60 PM CoV-2 viru ViroKey HT SARS-CoV-2 RT-PCR Test ViroKey Viru V ViroKey Viru V ViroKey Viru V			G2	1986HK_7				CoV-2 viru	
A3 HTPC,Re Test C0-2 viru B3 1980HK7 V'301087*PCRRX:000000002022:01-25 C0-2 viru C3 NC,Ref ID List ViroKey HT SARS-Co-2 RT-PCR Test C0-2 viru D3 HTP C,Re ID List Name ViroKey HT SARS-Co-2 RT-PCR Test C0-2 viru F3 1996HK7 ID Creation Date 4/22/2021 6 0 PM C0-2 viru F3 NC,Ref ID Creation Date CF9211K1 C0-2 viru v Lysis Kit V*301089*EXTRX*00000001*2022-01-31 2* 2*			H2	NC_Ref		7.07	CD	CoV-2 viru	
C3 NC,Ref ID List ViroKey HT SARS-CoV-2 RT-PCR Test coV-2 viru D3 HT PC,Ref ID List Name ViroKey HT SARS-CoV-2 RT-PCR Test coV-2 viru E3 1996HK,7 ID Creation Date 4/22/2014 coV-2 viru F3 NC,Ref ID List Name Cre3211K1 coV-2 viru Lysis Kit V*301099*EXTRX*000000012022-01-31 2 2			A3	HT PC_Re	Assay Ni		UN	CoV-2 viru	
D3 HTPC_Re ID List Name ViroKey HT SARS-CoV-2 RT-PCR Test DV-2 viru E3 1998HC7 ID Creation Date 4/22/2021 6.0 PM coV-2 viru coV-2 viru F3 NC_Ref Plate ID CF9211K1 coV-2 viru coV-2 viru Lysis Kit V*301089*EXTRX*000000001*2022-01-31 > coV-2 viru >			B3	1986HK_7		V*301087*PCRRX*0000000000*2022-01-25		CoV-2 viru	
63 1996H(7) ID Creation Date 4/22/2021 6.0 PM CV-2 viru F3 NC_Ret Plate ID CF9211K1 CV-2 viru Lysis Kit V*301089*EXTRX*000000001*2022-01-31 > >			C3	NC_Ref				CoV-2 viru	
F3 NC.Ref Plate ID CF9211K1 OV-2 viru v Lysis Kit V*301089*EXTRX*000000001*2022-01-31 >			D3	HT PC_Re	ID List Name	ViroKey HT SARS-CoV-2 RT-PCR Test		CoV-2 viru	
 Lysis Kit V*301089*EXTRX*000000001*2022-01-31 			E3	1986HK_7	ID Creation Date	4/22/2021 6:0 PM		CoV-2 viru	
Lýsis kit V 301069 EARA 000000001 2022-01-31			F3	NC_Ref	Plate ID	CF9211K1			
SentosaSX SN SNF167		<			Lysis Kit	V*301089*EXTRX*000000001*2022-01-31		>	
					SentosaSX SN	SNF167			
About Contact Us Help << Previous Comments Services SADVare Comments:	bout Contact Us	Help	<< Previou	ıs	Comments			~	

Click "Preview Report" to preview the report.

- 3.2. The "Report Preview" pop-up window will be displayed. The report can be saved, printed and / or exported to PDF or Excel.
 - Click the "
 - The *"Save As"* pop-up window will be displayed. Select a location and click *"Save"* to save the report.

Sentosa SA201 Reporter				* Report Preview				- 6
	Sa	mples	1	H 4 1 of 2 🕨 🔰 🔶 👘		Next		-
Test Names Crokey SPAS-Coll-3	E Filt	er Target:	All Targets	Sentosa [®] SA201		VE	-	Preview Report
IT KEPCR TOU	Colo	r Well	▲ Sample	ViroKey HT SARS-CoV-2 RT-PC		^	Experimental Information	
		A1	V*202258*			d	d to PC.	Quantitation Information
Operator:		B1	V*202259	Experiment Inform	nation	d t	to Samp	Cycling Profile
		C1	1986HK_7			Co	V-2 viru	Raw/ Normalized Curve
		D1	NC_Ref	Run ID	ViroKey HT SARS-CoV-2 RT-PCR Test 20210416 HT ReagentStability Run01	Co	V-2 viru	Message
		E1	HT PC_Re	User	Sentosa SA201 Series Software User: INSTR-ADMIN	Co	V-2 viru	
Template:		F1	1986HK_7		Sentosa SA201 Reporter User: INSTR-ADMIN	Co	V-2 viru	
Wolkey SARS-Cold-1		G1	NC_Ref	Run Signature	ON	Lo.	V-2 viru	
HT RT OCR Test add		H1	HT PC_Re	Run Start	16-April-2021 17:54:58	Co	V-2 viru	
Notes		A2	1986HK_7	Last Modified	23-April-2021 16:26:23	Lo.	V-2 viru	
Notes		B2	NC_Ref	Software Version	Sentosa SA201 Series Software Version: v1.0.1	Co	V-2 viru	
		C2	HT PC_Re		Sentosa SA201 Reporter Version: 1.5.0003	Co	V-2 viru	
		D2	1986HK_7		Assay Package Version: 1.6.0017 RUO	Co	V-2 viru	
		E2	NC_Ref	Instrument Type	Sentosa SA201	Co	V-2 viru	
		F2	HT PC_Re	Lot No. of SARS-CoV-2-HT M1	TX0004	Lo.	V-2 viru	
		G2	1986HK_7	PCR Volume	26uL	Lo.	V-2 viru	
		H2	NC_Ref	Assav Kit	Sentosa SA201 ViroKey SARS-CoV-2 HT RT-PCR Test PCR	Lo.	V-2 viru	
		A3	HT PC_Re	hoody fut	Test	Co	V-2 viru	
		B3	1986HK_7		V*301087*PCRRX*0000000000*2022-01-25	Co	V-2 viru	
		C3	NC_Ref	ID List	ViroKey HT SARS-CoV-2 RT-PCR Test	Co	V-2 viru	
		D3	HT PC_Re	ID List Name	ViroKey HT SARS-CoV-2 RT-PCR Test	Co	V-2 viru	
		E3	1986HK_7	ID Creation Date	4/22/2021 6:0 PM	Co	V-2 viru	
		F3	NC_Ref	Plate ID	CF9211K1	Co	V-2 viru 🛩	
	<			Lysis Kit	V*301089*EXTRX*000000001*2022-01-31		>	
				SentosaSX SN	SNF167			
bout I Contact Us I	Help	<< Previo	us	Comments	Sentosa SA201 Series Software Comments: Sontosa SA201 Departer Notos:	. ~		

tosa SA201 Reporter	* Report Preview				- 6
	Samples	N +®® ₿⊡¤N- 10	0% · Find	Next	-
Names	Filter Tan Save As	A State States		×	Preview Report
ley SAMS-CENEE I-PCR Test	Color We	1 Test Data > SARS-CoV-2 HT > smp	ע ע גע אין	ch smp	Experimental Information
ator:	A1 Organize New folder B1			H • 🕐	Quantitation Information Cycling Profile
	C1 This PC	^ Name	 Date modified 	Туре	Raw/ Normalized Curve
	D1 3D Objects	ViroKey HT SARS-CoV-2 RT	PCR Test_202 7/27/2021 10:03 AM	Microsoft Edge P	Message
	F1 Documents				
	G1 Documents				
	A2 Music				
	B2 Pictures				
	C2 shared_folder on min-koko-macbo				
	D2 Videos				
	E2 E2				
	F2				
	H2 Google Drive (G:)				
	B3 Network	v <		,	
	C3		1.15 8 94 14		
		V-2 RT-PCR Test_20210416_HT_ReagentSt	ability_Run01.pdf	~	
	E3 Save as type: PDF (*.pdf)			Y	
	F3				
	A Hide Folders		Save	Cancel	
i <u>Contact Us</u> i He	clp. << Previous Comments	Sentosa SA201 Series Sentosa SA201 Perce		*	-

For Prescription Use Only

After the run is complete, proceed to *"Result interpretation"* on page 11. **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.

A reservoir collects liquid waste generated during the nucleic acid extraction procedure. Dispose the liquid waste according to the local safety and environment regulations. Dispose the biohazard bags after each run.

Perform regular cleaning of the Hamilton Microlab[®] STAR[™] and the *Sentosa*[®] SA201 after each run. Refer to the respective instrument maintenance videos or user manuals 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, 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 user manual 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 reage	nts of the ViroKey [®] HT Virus Total Nucleic Acid
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 value	es observed for samples
a)	Magnetic beads were not completely re-suspended	Mag (magnetic beads) requires thorough vortexing before use to ensure proper resuspension.
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 Buffer D1 (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.

For Prescription Use Only

e)	Clogging of pipette	Insoluble material was not removed from the sample
	tip due to insoluble	prior to starting the extraction procedure on the
	material in the	Hamilton Microlab [®] STAR [™] instrument. To remove
	samples	insoluble material, centrifuge the diluted sample
		suspension at 3,000 x g for 1 minute, and transfer the
		supernatant to a fresh sample tube.

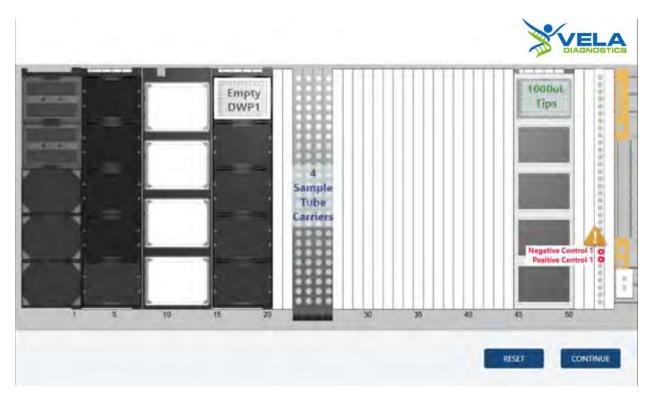
- 4. No signal with positive control (PC) in the Green, Orange or Red fluorescence channels
- a) PCR conditions do not comply with the protocol Ensure that the correct thermal cycling conditions are input into the *Sentosa*[®] SA201 Series Software.
- b) Incorrect PCR Ensure that the correct thermal cycling conditions are input into the *Sentosa*[®] SA201 Series Software.
- 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
 c) Incorrect papering
 c) Incorrect papering
- e) Incorrect passive reference setting is set correctly to reference setting ROX in the well inspector and reanalyze.
- f) No ROX added to Repeat PCR. PCR master mix
- 5. Signals with the negative control in the Green and Orange fluorescence channels of the analytical PCR
- a) Contamination Repeat the extraction and PCR protocols with new reagents.
- up Ensure that the workspace and instruments are decontaminated as recommended.

First check the Rn for true amplification profile and rule out baseline issues. See "Automated data analysis", page 60 .

b) Baseline issue Check the raw (Rn) signal of the green channel of the NC. Sudden jumps in baseline can become false positive call in the dRn.

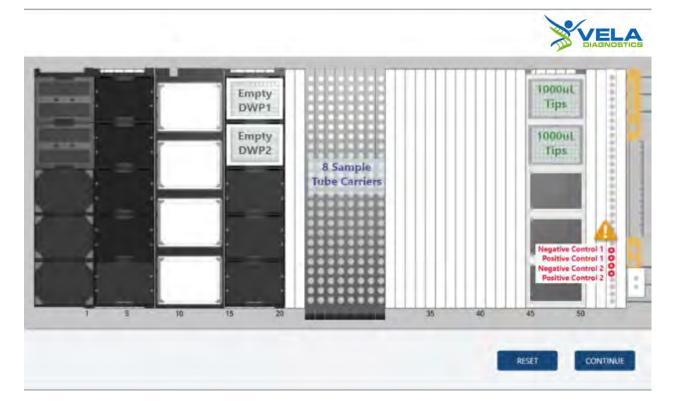
Appendix for Sample Plate Preparation

Appendix A: Layout of the Hamilton Microlab[®] STAR[™] platform for sample plate preparation (with 1 deepwell plate) using STAR8AL96 Vela_SampleTransfer_V1.2.med application



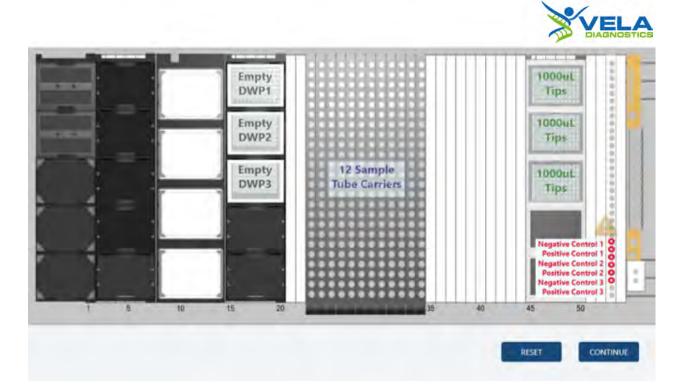
Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	HT U Deepwell Plate, Barcoded, 2.2 mL
23 to 26	4 Sample Tube Carriers
45 to 50	HT Conductive 1 mL Filter Tips (96)
53	NC & PC (Positions 25 and 26)

Appendix B: Layout of the Hamilton Microlab[®] STAR[™] platform for sample plate preparation (with 2 deepwell plates) using STAR8AL96 Vela_SampleTransfer_V1.2.med application



Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	2x HT U Deepwell Plate, Barcoded, 2.2 mL
23 to 30	8 Sample Tube Carriers
45 to 50	HT Conductive 1 mL Filter Tips (2x96)
53	2x NC & 2x PC (Positions 25 to 28)

Appendix C: Layout of the Hamilton Microlab[®] STAR[™] platform for sample plate preparation (with 3 deepwell plates) using STAR8AL96 Vela_SampleTransfer_V1.2.med application



Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	3x HT U Deepwell Plate, Barcoded, 2.2 mL
23 to 34	12 Sample Tube Carriers
45 to 50	HT Conductive 1 mL Filter Tips (3x96)
53	3x NC & 3x PC (Positions 25 to 30)

Appendix D: Layout of the Hamilton Microlab[®] STAR[™] platform for sample plate preparation (with 4 deepwell plates) using STAR8AL96 Vela_SampleTransfer_V1.2.med application

1000uL		Empty	
Tips		DWP1	· · · · ·
1000ut. Tips		Empty DWP2	
1000uL	16 Sample Tube Carriers	Empty DWP3	
1000ut		Empty	
Tios		DWP4	
Negative Control 1 Positive Control 1 Negative Control 2 Positive Control 2 Negative Control 3		: :	
Positive Control 3 O	40	15 20	1 5 10

Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	4x HT U Deepwell Plate, Barcoded, 2.2 mL
23 to 38	16 Sample Tube Carriers
45 to 50	HT Conductive 1 mL Filter Tips (4x96)
53	4x NC & 4x PC (Positions 25 to 32)

.....

Appendix for Viral RNA Extraction

Appendix E: Layout of the Hamilton Microlab[®] STAR[™] platform for viral RNA extraction (with 1 deepwell plate) using STAR8AL96 Vela_ViroKey_V3.10.med application

Tips			DWP1	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	Lysis
000uL Tips									Beads
Buffer D2		The second second							
Buffer D3									
	Buffer D4					1000uL Tips	50uL Tips		

Track(s)	Description
1	 HT Conductive 1 mL Filter Tips (2x96) 2x HT 300 mL Reservoir
2 to 7	HT 300 mL Reservoir
8 to 14	Empty
15 to 20	HT U Deepwell Plate, Barcoded, 2.2 mL
21 to 26	HT Conductive 1 mL Filter Tips (96)
27 to 32	HT Conductive 1 mL Filter Tips (96)
33 to 38	HT Conductive 1 mL Filter Tips (2x96)
39 to 44	 HT Conductive 1 mL Filter Tips (96) HT Conductive 50 µL Filter Tips (96)
45 to 50	HT Conductive 1 mL Filter Tips (96)
52	2x HT Reagent Tub with Lid, 60 mL

Appendix F: Layout of the Hamilton Microlab[®] STAR[™] platform for viral RNA extraction (with 2 deepwell plates) using STAR8AL96 Vela_ViroKey_V3.10.med application

000uL Tips			DWP1	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	
000uL Tips			DWP2	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	
uffer D2									000000
uffer D3									
uffer D3	Buffer D4		Beads	Lysis	1000uL Tips	1000uL Tips	50uL Tips		
1	5	10	15 20) 25	30	35	40	45 50	

Track(s)	Description
1	HT Conductive 1 mL Filter Tips (2x96)
	3x HT 300 mL Reservoir
2 to 7	HT 300 mL Reservoir
8 to 14	Empty
15 to 20	2x HT U Deepwell Plate, Barcoded, 2.2 mL
15 10 20	HT 300 mL Reservoir
21 to 26	HT Conductive 1 mL Filter Tips (2x96)
211020	HT 300 mL Reservoir
27 to 32	HT Conductive 1 mL Filter Tips (3x96)
33 to 38	HT Conductive 1 mL Filter Tips (3x96)
39 to 44	HT Conductive 1 mL Filter Tips (2x96)
	 HT Conductive 50 µL Filter Tips (96)
45 to 50	HT Conductive 1 mL Filter Tips (2x96)

Appendix G: Layout of the Hamilton Microlab[®] STAR[™] platform for viral RNA extraction (with 3 deepwell plates) using STAR8AL96 Vela_ViroKey_V3.10.med application

		-							AGNOSTIC
000uL Tips			DWP1	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	000000
000uL Tips			DWP2	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	
Buffer D2		[DWP3	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	0.000
Buffer D3									
Buffer D3	Buffer D4		Beads	Lysis	1000uL Tips	1000uL Tips	50uL Tips		
1	5	10	15 20	25	30	35	40	45 50	

Track(s)	Description
1	 HT Conductive 1 mL Filter Tips (2x96) 3x HT 300 mL Reservoir
2 to 7	HT 300 mL Reservoir
8 to 14	Empty
15 to 20	 3x HT U Deepwell Plate, Barcoded, 2.2 mL HT 300 mL Reservoir
21 to 26	HT Conductive 1 mL Filter Tips (3x96)HT 300 mL Reservoir
27 to 32	HT Conductive 1 mL Filter Tips (4x96)
33 to 38	HT Conductive 1 mL Filter Tips (4x96)
39 to 44	 HT Conductive 1 mL Filter Tips (3x96) HT Conductive 50 µL Filter Tips (96)
45 to 50	HT Conductive 1 mL Filter Tips (3x96)

Appendix H: Layout of the Hamilton Microlab[®] STAR[™] platform for viral RNA extraction (with 4 deepwell plates) using STAR8AL96 Vela_ViroKey_V3.10.med application

000uL Tips			DWP1	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	000000
000uL Tips			DWP2	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	
uffer D2			DWP3	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	0000000
uffer D3			DWP4	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	1000uL Tips	
uffer D3	Buffer D4		Beads	Lysis	1000uL Tips	1000uL Tips	50uL Tips		
1	5	10	15 20	25	30	35	40	45 5	

Track(s)	Description
1	 HT Conductive 1 mL Filter Tips (2x96) 3x HT 300 mL Reservoir
2 to 7	HT 300 mL Reservoir
8 to 14	Empty
15 to 20	 4x HT U Deepwell Plate, Barcoded, 2.2 mL HT 300 mL Reservoir
21 to 26	 HT Conductive 1 mL Filter Tips (4x96) HT 300 mL Reservoir
27 to 32	HT Conductive 1 mL Filter Tips (5x96)
33 to 38	HT Conductive 1 mL Filter Tips (5x96)
39 to 44	 HT Conductive 1 mL Filter Tips (4x96) HT Conductive 50 µL Filter Tips (96)
45 to 50	HT Conductive 1 mL Filter Tips (4x96)

Appendix I: Consumable summary for 1 deepwell plate

-	1mL Tips	50uL Tips	2.2mL 96-well DWP	60mL Trough	300mL Trough
LABWARE			0	2	
QTY	8	1	1	2 Lysis 40 ml Beads 40 ml	Buffer D2 80 m Buffer D3 125 m Buffer D4 25 m
					1.1.1.1

Appendix J: Consumable summary for 2 deepwell plates

	1mL Tips	50uL Tips	2.2mL 96-well DWP	60mL Trough	300m	L Troug	h
LABWARE			0	2			
QTY	14	1	2	0	6		
					Lysis Beads Buffer D2 Buffer D3A Buffer D3B Buffer D4	80 80 160 125 125 50	

Appendix K: Consumable summary for 3 deepwell plates

-	1mL Tips	50uL Tips	2.2mL 96-well DWP	60mL Trough	300mL	Trough	h
LABWARE			0	0			
QTV	19	1	3	0	6	5	_
						120 120 240 187.5 187.5 50	

Appendix L: Consumable summary for 4 deepwell plates

-	1mL Tips	50uL Tips	2.2mL 96-well DWP	60mL Trough	300m	L Troug	h
LABWARE			0	2			
QTY	24	1	4	0		6	
					Lysis Beads Buffer D2 Buffer D3A Buffer D3B Buffer D4	160 160 300 250 250 50	n n n n

Appendix for HT PCR setup

Appendix M: Layout of the Hamilton Microlab[®] STAR[™] platform for HT PCR setup (with 1 sample plate) using STAR8AL96 Vela_PCRsetup_V1.3.med application

•	DWP1	PCR Plate 1	50uL Tips	1	K - Y	1	
					<u> </u>		
				1000uL Tips			
		1 PCR Strip	300uL Tips				PCR O MM

Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	HT U Deepwell Plate, Barcoded, 2.2 mL
21 to 26	 MicroAmp[®] Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL 1x HT 8-Strip Tubes, Clear, 0.2 mL
27 to 32	 HT Conductive 50 μL Filter Tips (96) HT Conductive 300 μL Filter Tips (96)
33 to 38	HT Conductive 1 mL Filter Tips (96)
39 to 44	Empty
45 to 50	Empty
53	PCR MM (Position 29)

Appendix N: Layout of the Hamilton Microlab[®] STAR[™] platform for HT PCR setup (with 2 sample plates) using STAR8AL96 Vela_PCRsetup_V1.3.med application

			PCR	50uL				
•		DWP1	Plate 1	Tips				
	Tomas a	DWP2	PCR Plate 2	50uL Tips				
					1000uL Tips			
			2 PCR Strips	300uL Tips				PCR 8
1 5	10	15 20	25	30	35	40	45	50

Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	2x HT U Deepwell Plate, Barcoded, 2.2mL
21 to 26	 2x MicroAmp[®] Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL 2x HT 8-Strip Tubes, Clear, 0.2 mL
27 to 32	 HT Conductive 50 µL Filter Tips (2x96) HT Conductive 300 µL Filter Tips (96)
33 to 38	HT Conductive 1 mL Filter Tips (96)
39 to 44	Empty
45 to 50	Empty
53	2x PCR MM (Positions 29 and 30)

Appendix O: Layout of the Hamilton Microlab[®] STAR platform for HT PCR setup (with 3 sample plates) using STAR8AL96 Vela_PCRsetup_V1.3.med application

Second Second			1	1			
	DWP1	PCR Plate 1	50uL Tips				
	DWP2	PCR Plate 2	50uL Tips				
	DWP3	PCR Plate 3	50uL Tips				
				1000uL Tips			
		3 PCR Strips	300uL Tips				PCR O MM O
1 5 10	15 20) 25	30	35	40	45	50

Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	3x HT U Deepwell Plate, Barcoded, 2.2mL
21 to 26	 3x MicroAmp[®] Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL 3x HT 8-Strip Tubes, Clear, 0.2 mL
27 to 32	 HT Conductive 50 µL Filter Tips (3x96) HT Conductive 300 µL Filter Tips (96)
33 to 38	HT Conductive 1 mL Filter Tips (96)
39 to 44	Empty
45 to 50	Empty
53	3x PCR MM (Positions 29 to 31)

Appendix P: Layout of the Hamilton Microlab[®] STAR[™] platform for HT PCR setup (with 4 sample plates) using STAR8AL96 Vela_PCRsetup_V1.3.med application

			200000000000					
•		DWP1	PCR Plate 1	50uL Tips				
	[DWP2	PCR Plate 2	50uL Tips		_		
		DWP3	PCR Plate 3	50uL Tips				
		DWP4	PCR Plate 4	50uL Tips	1000uL Tips			
			4 PCR Strips	300uL Tips				PCR O MM O
1 5	10	15 20	25	30	35	40	45	50

Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	4x HT U Deepwell Plate, Barcoded, 2.2mL
21 to 26	 4x MicroAmp[®] Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL 4x HT 8-Strip Tubes, Clear, 0.2 mL
27 to 32	 HT Conductive 50 µL Filter Tips (4x96) HT Conductive 300 µL Filter Tips (96)
33 to 38	HT Conductive 1 mL Filter Tips (96)
39 to 44	Empty
45 to 50	Empty
53	4x PCR MM (Positions 29 to 32)

References

- 1) Mackay, I.M. (2004). Real-time PCR in the microbiology laboratory. *Clin Microbiol Infect.* 10(3), 190–212.
- 2) Pyrc, K., Berkhout B. and van der Hoek L. (2006). The Novel Human Coronaviruses NL63 and HKU1. *J Virol*. 81(7), 3051-3057.
- 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.
- 4) 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.
- 5) 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.
- 6) 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.
- 7) Miriam E.R. Darnell, K. S. (2004). Inactivation of the coronavirus that induces severe acute respiratory syndrome, SARS-CoV. *Journal of Virological Methods 121 (2004) 85–91*, 87.

For use under an Emergency Use Authorization Only

For Prescription Use Only

For more information, kindly contact your Vela Diagnostics representative. © 2021 Vela Diagnostics Holding Pte Ltd. All rights reserved. OncoKey[®], PathoKey[®], VELA[®] and ViroKey[®] are registered trademarks of Vela Diagnostics Holding Pte Ltd in several markets including the US, EU, PRC and the UK. Sentosa[®] is a registered trademark of Vela Diagnostics Holding Pte Ltd outside Singapore in several markets including the US, EU, PRC and the UK. All Sentosa[®] products mentioned above are by Vela Diagnostics.

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the ViroKey[®] HT SARS-CoV-2 RT-PCR Test to the following terms:

- 1. The ViroKey[®] HT SARS-CoV-2 RT-PCR Test may be used solely in accordance with the ViroKey[®] HT SARS-CoV-2 RT-PCR Test user manual and for use with components contained in the test only.
- 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[®] HT SARS-CoV-2 RT-PCR Test user manual and additional protocols.
- 3. Other than expressly stated licenses, Vela Diagnostics makes no warranty that this kit and / or its use(s) do not infringe the rights of third parties.
- 4. This kit and its components are licensed for one-time use and may not be re-used, re-furbished or re-sold unless otherwise specified in this document.
- 5. Vela Diagnostics specifically disclaims any other licenses, expressed or implied other than those expressly stated.
- 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.
- 7. Vela Diagnostics may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, please contact the authorized Vela Diagnostics representative.

© 2021 Vela Diagnostics Holding Pte Ltd. All rights reserved.

2021-09-30.

For use under an Emergency Use Authorization Only

For Prescription Use Only

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