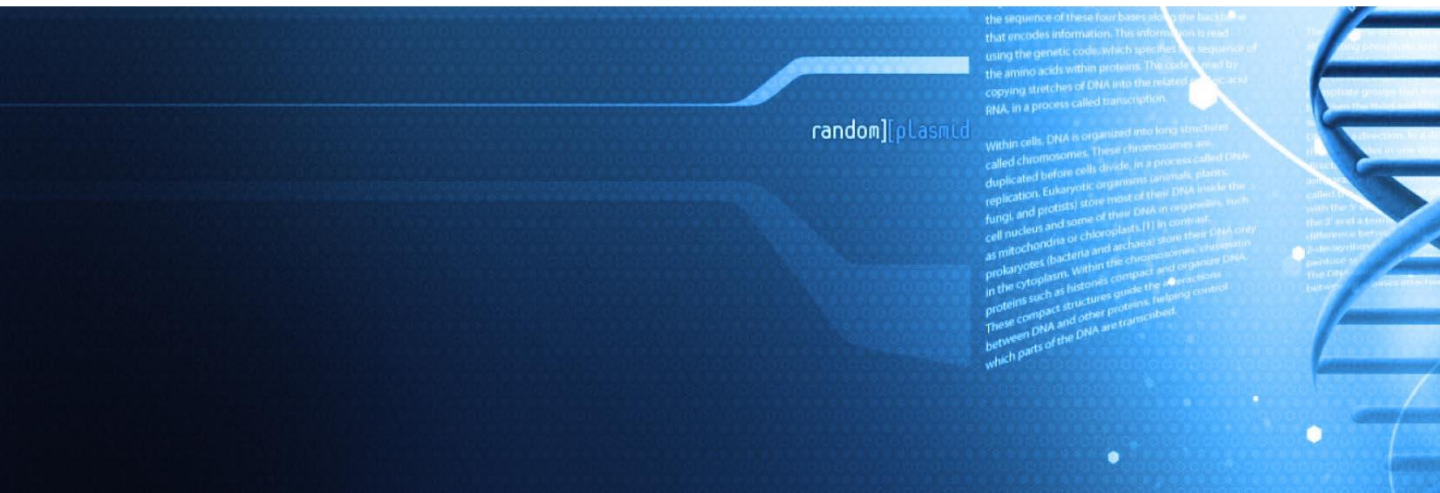
Techcomp

Sample Preparation

Kurabo / XXpress / Major Science / SERVA / TOMY / BIO-HELIX

COVID-19 RNA Purification and Detection

For every sample, RNA type, and application



Speed is everything

Rapid RNA isolation is a crucial step to understand gene expression levels and detection of the COVID-19 Virus .

With all the aid Techcomp Limited has to offer, you can be confident that you're getting started on the right foot. We'll be there to support you with world best RNA purification kits, trusted RNA tools, and experienced technical support, all backed by over 30 years of leadership and innovation in Molecular Biology technologies.

- *Isolate from any sample type, for any application.*
- *Obtain high-purity, intact RNA.*
- *Achieve high yields, even from small sample quantities.*

Sample collection 1

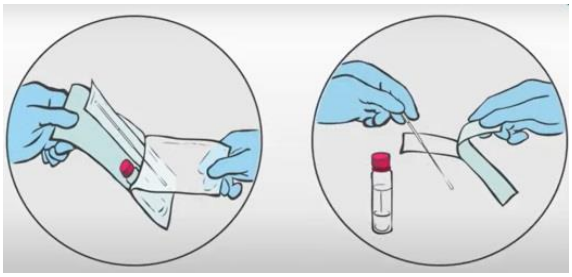
Sample collection

Careful attention must be paid during the sample collection process to ensure the safety of the collecting personnel. Inactivate the sample as soon as the sample is collected (virus lysis buffer pre-packed in the collection container), and make sure packaging is in strict accordance with regulations before transport to the testing facility.

This nucleic acid test method is applicable to nucleic acid samples taken from samples such as throat swabs, nasopharyngeal swabs, sputum fluids, alveoli irrigation fluids, urine, feces, etc. Samples for virus isolation and nucleic acid testing should be processed as soon as possible. Samples that can be detected within 24 hours can be stored at 4°C, and samples that cannot be detected within 24 hours should be stored at minus 70°C or below (if there is no minus 70°C storage, temporary storage at minus 20°C can suffice).

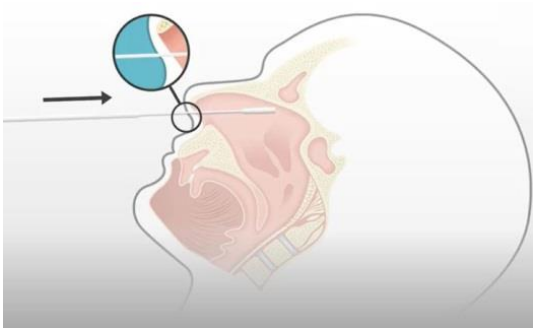
Note: Proper collection of samples is essential for the accuracy of the test. The following instructions pertain to throat swabs and are for reference only: a sample collection kit will be provided for each user. The kit includes a sterilized sample collection rod, a sample collection tube containing 0.9ml virus lysis buffer, a registration form to fill out personal information, and an instruction manual for the required procedure specific to the test type. If sputum, feces, or urine samples need to be collected, there will be an additional larger sample collection container with a corresponding label. Users need to bring their own disinfection solution, 75% alcohol or 10% bleach, garbage bags, etc. The swab sample must be collected with a plastic rod swab with a synthetic material swab head (e.g. polypropylene fiber) to avoid inhibition of nucleic acid detection.

① Wash Hands and put on any personal protection equipment;



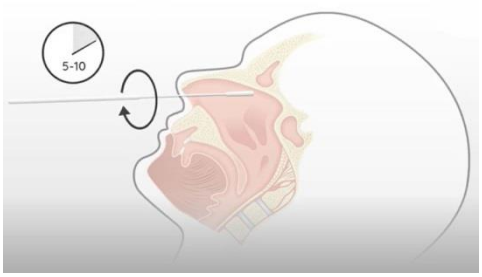
② Open the plastic sealed pouch in the sample collection kit and remove all the items inside;

③ Tear open the paper bag packaging of the sample collection stick, remove the collection stick, and collect a sample with the sterile swab using its finer end;

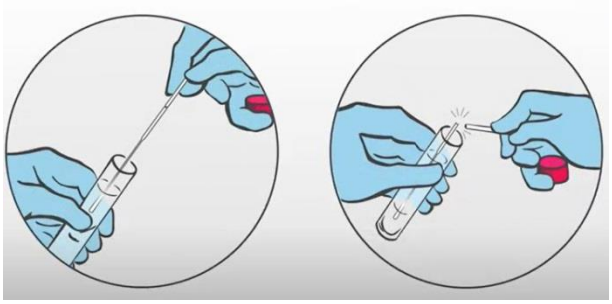


④ Insert swab into one nostril straight back, not upwards and horizontally to the nasopharynx until resistance is met.

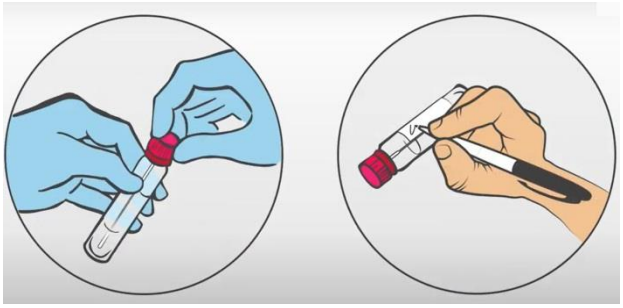
Sample collection 2



⑤ Rotate the swab up to five times and hold in place for 5-10 seconds to collect sample materials

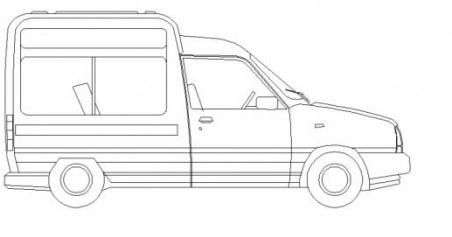


⑥ Insert the swab into the viral transport medium and ensure the swab heads are fully immersed in the solution. Then break handle at breakpoint line.

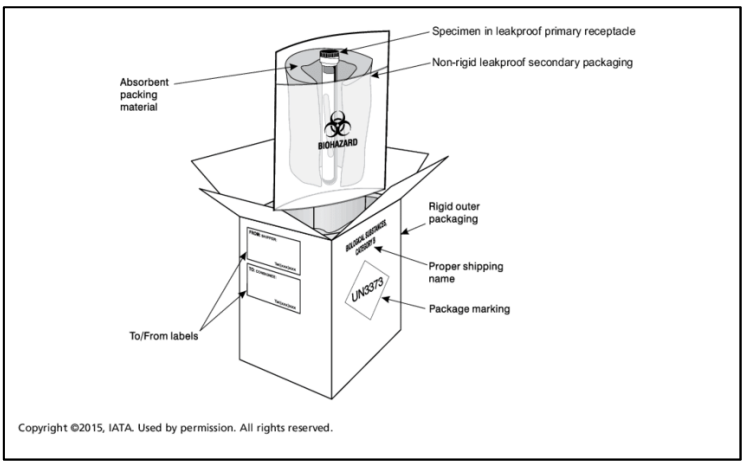


⑦ Tighten the tube cap to ensure that it is sealed; than shake the collection tube up and down 5 times to mix thoroughly.

⑧ Put the collection tube in the original pouch and seal the mouth of the bag; Wipe or spray the surfaces of the pouch with a disinfectant;



⑨ Store the sample at room temperature for collection and transport;



Biohazard transport packaging illustration

Pre-experimental preparation 1

Whole laboratory 7.5 % H2O2 sterilization for at least 20min

Hydrogen peroxide concentration	Mycobacterial	Disinfection Time >99.9% inactivation	Stress test	Recommend H202 exposure
7.5%	Bacteria (Geobacillus stearothermophilus)	10 minutes	14 days	3 minutes
7.5%	Yeasts	10 minutes	14 days	5 minutes
7.5%	Fungi	30 minutes	14 days	5 minutes
7.5%	Viruses	30 minutes	14 days	5 minutes
7.5%	Spores	30 minutes	14 days	20 minutes
7.5%	M. tuberculosis	10 minutes	14 days	10 minutes

GUARDiON
CLEAN & SAFE

GUARDiON system Specifications	
Model	**GUARDiON H2T**
Main Unit dimensions (W x D x H)	45cm X 45cm X 95cm
Main Unit weight	56kg
Sub Unit dimensions (W x D x H)	45cm X 45cm X 107cm
Sub Unit weight	52kg
Input voltage	110V / 220V

Protocol Recommended by

醫院管理局 HOSPITAL AUTHORITY

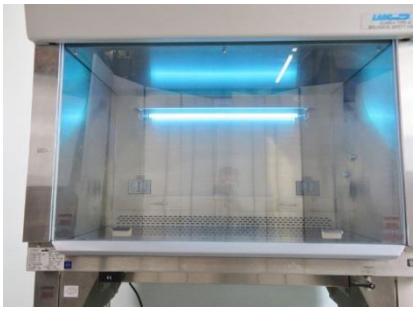
CDC

사단법인 대한병원협회 Korean Hospital Association

CDC Centers for Disease Control and Prevention National Center for Health Statistics

Pre-experimental preparation 2

The required materials are placed in a biosafety cabinet, and subjected to 30min UV



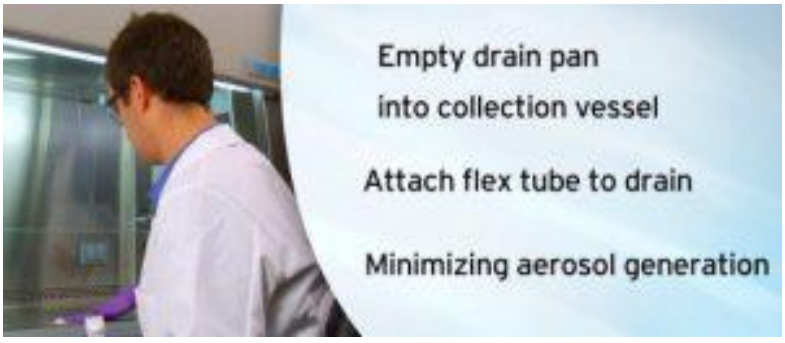
Pre-experimental preparation 3

After UV sterilization, all relevant equipment must be disinfected with 70% Alcohol

Decontamination of your Nuaire Biological Safety Cabinet (BSC) is important for many reasons, such as keeping you and your laboratory safe from harmful toxins and spills while working in the BSC, and also for the validity and quality of your experiments. In this article, we have outlined 10 of the most important Decontamination steps which should be performed after every use:

1. Enclose any items which have been in contact with the agent and cover any waste containers.
2. Allow the cabinet to operate for 5 minutes with no activity, which should purge airborne contaminants from the work area.
3. Clean/decontaminate all containers and equipment and then remove from the cabinet.
4. Once the cabinet is empty, you should allow 20-30 minutes to thoroughly decontaminate all interior work surfaces, making sure to clean the back and side of the cabinet, and the interior of the glass. This may take more or less time depending on the disinfectant and the microbiological agent used.
5. If using a chloride type disinfectant, after contact time, wipe down interior surfaces with a 70% alcohol solution to protect stainless steel interiors from corrosion.
6. The drain pan should be emptied into a collection vessel containing disinfectant; and the drain valve can be disinfected using a flexible tube (see video below)
7. Any spilled fluid and disinfectant solution on the work surface should be absorbed with paper towels and discarded into a biohazard bag.
8. Hands should be washed whenever gloves are changed or removed.
9. If necessary, the cabinet should also be monitored for radioactivity and decontaminated when necessary.
10. The cabinet can be turned off and the vertically sliding window closed. If desired, the Ultra Violet (UV) light may be turned on. The Centers for Disease Control (CDC), National Institute of Health (NIH), and National Science Foundation (NSF) all agree that UV lamps are neither recommended, nor required in Biological Safety Cabinets; however, if you are going to use one please follow the safety guides below to ensure you and your lab's safety:

- The room should never be occupied when UV lamp is in use (UV light will damage the human eye and skin very quickly).
- Vertical sliding window must be closed. If it has a hinged window, use manufactures enclosure panel.
- Lamp must be wiped free of dust/linT weekly.
- UV wavelength must be checked periodically.
- Thoroughly wash your hands and arms with warm, soapy water. Personnel should remove their gloves and gowns and wash their hands as the final step in safe microbiological practices



Steps for RNA Isolation 1

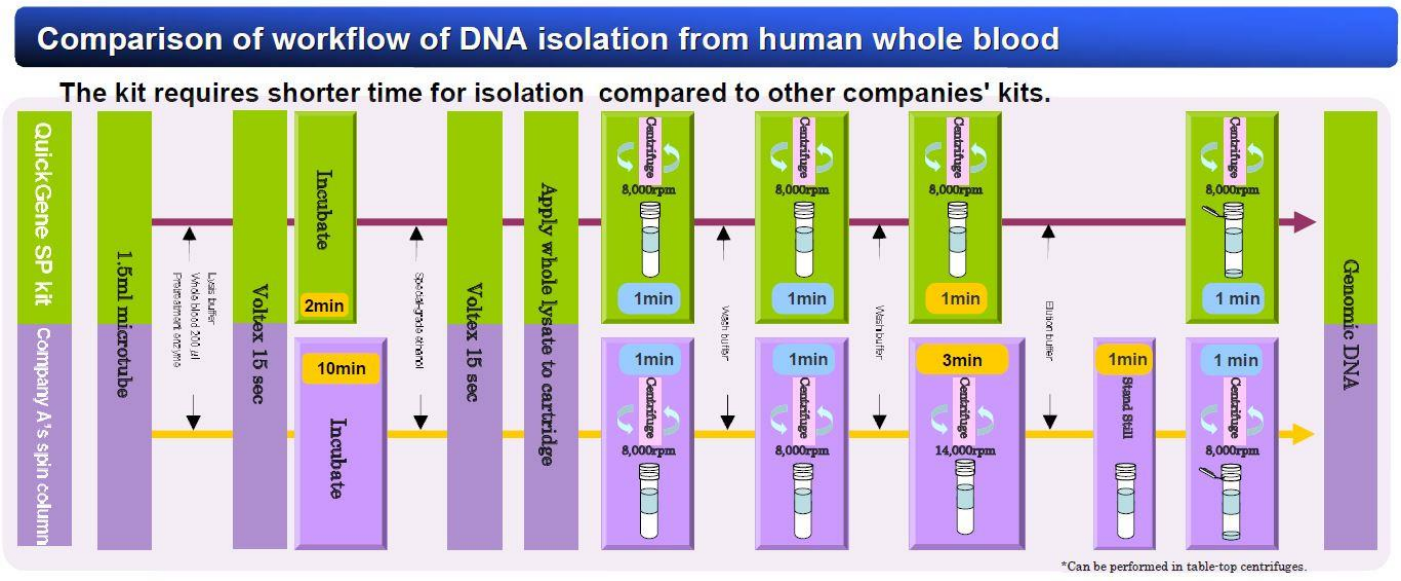
Nucleic acid extraction

Viral RNA extraction can be done using Kurabo Quick Gene SP kit RA-b-1,2,8 & RG-16 or other RNA extraction kits, following the kit instructions. Any remaining extracted samples not used for testing are placed as soon as possible in minus 70 C or below for storage (if there is no minus 70 C storage condition, store in the refrigerator at minus 20 C).

QuickGene

For Spin-cartridge method isolation

Kurabo Quick Gene SP kit



Steps for RNA Isolation 2

Centrifugation at 8,000 rpm for 1 minutes is require for RNA isolation.
Recommendation model for such procedure.

Dynamica

VELOCITY 15μ SPECIFICATIONS	Velocity 15μ
Maximum Speed	15,000 RPM
Maximum RCF	21,380 x g
Run Time	30 seconds to 99 minutes or HOLD (Continuous)
Rotor (Standard)	24 x 1.5/2.0 ml tubes
Rotor (Optional)	18 x 5 ml tubes, 36 x 0.5 ml tubes, 4 x 0.2ml 8-strip PCR tubes
Noise Level	≤ 58dB
Dimensions (w x d x h)	280 x 364 x 266 mm
Weight	12 kg
Power Supply	AC 200-240V 50/60Hz or AC 110-120V 50/60Hz

VELOCITY 15HR Specifications

MODEL	VELOCITY 15HR
Maximum Speed	15,000 RPM
Maximum RCF	21,500 x g
Maximum capacity	2 ml x 24 and 0.5 ml x 24
Rotor (Standard)	24 x 1.5/2.0 ml tubes
Rotor (Optional)	24 x 0.5/1.5/2.0 ml tubes, 32 x 0.2ml PCR tubes
Speed Control Range (rpm)	300 to 15,000 (in increments of 100 rpm)
Temperature range adjustment	-20°C (displayed "Lo") to 40°C
Timer	1 to 99 minutes (in increments of 1 minute.) With a HOLD function(continuous operation)
Acceleration/deceleration time variable	2-stage variable acceleration, 2-stage braked deceleration plus free deceleration
Drive motor	Brushless DC motor (inverter control)
Memory-based programmed operation	SPEED/RCF, TIME, TEMP, ACCEL,DECEL and stop melody
Rotor stop signal	Selectable from 5 types of stop melodies, beep and mute
Safety devices	Door interlock, dual overspeed detector, imbalance detector, abnormal motor temperature deterctor, etc.
Compliance with standards	CE marking(EN61326,61010-1,61010-2-020)
Heat radiation	500W
Power requirements	AC120V, 60Hz 15A AC220/230V,50/60 Hz 10A
Dimensions	290 (W) × 520 (D) × 300 (H)
Weight	40KG

Rotors Selection

FAS15E
(Standard)

FAS15F
(Option)

FAS15G
(Option)

Obtained purified RNA sample for following step

Steps for RNA Isolation 3

Experimental materials and reagent formulations

Recommend to use BioHelix LifeDireX - QP019-0100 COVID-19 RT-qPCR Detection Kit which include the following:

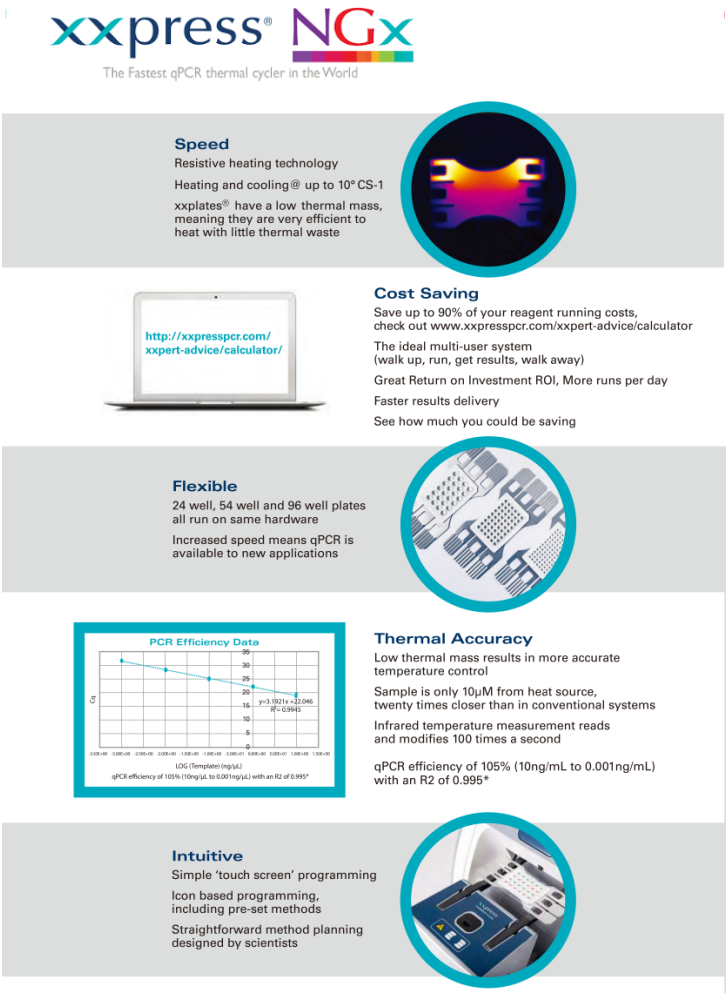
BIO-HELIX CO., LTD.

Part No.	Component	Volume	Reactions/Kit
QP019-0100-1	2X RT-qPCR Master Mix	1.25ml	100
QP019-0100-2	RT-qPCR Enzyme Mix	40 ul	100
QP019-0100-3	COVID-19 Primers	100 ul	100
QP019-0100-4	COVID-19 Probes	100 ul	100
QP019-0100-5	Positive Control Template	100 ul	20
QP019-0100-6	Negative Extraction Control	1.0 ml	20

Required Materials

》Real-Time PCR tubes 》Real-Time PCR instrument 》Nuclease–Free H2O

Real-Time PCR instrument recommend XXPRESS, RAMP rate 10C / sec



Steps for RNA Isolation 4

Nuclease–Free H2O, recommend Evoqua TYPE I water system (pervious known as Siemens water system) . The system can provide the most reliable TYPE I (Nuclease- Free water) for RNA isolation

Specification

		Ultra Clear TP TWF UV TM	Ultra Clear TP TWF UV UF TM
Pure water specifications			
Conductivity	µS/cm	2	2
Bacteria	cfu/ml	< 30	< 30
Ultra pure water specifications			
Delivery flow rate	l/min	1.8	1.8
Conductivity	µS/cm	0.055	0.055
Resistivity	MΩ-cm	18.2	18.2
TOC	ppb	< 1 - 3	< 1 - 3
DNase, RNase, DNA		-	free
Bacteria	cfu/ml	< 0.1	< 0.1
Endotoxins	EU/ml	< 0.001	< 0.001
Particles > 0.2 µm	per ml	< 1	< 1
Feed water specifcations			
Feed water pressure	bar	0.1 – 5	0.1 – 5
Conductivity	µS/cm	< 2000*	< 2000*
CO_2	mg/l	15	15
Silt density index	SDI	< 12*	< 12*
Free chlorine	mg/l	< 0.5*	< 0.5*
Total iron	mg/l	< 0.1	< 0.1
Temperature	°C	5 - 35	5 - 35
Shipping weight 30 l/60 l	kg	43/46	44/47
Power supply	V/Hz	100-240/50-60	100-240/50-60
Dimensions 30 l (H x W x D)	mm	530 x 560 x 320	530 x 560 x 320
Dimensions 60 l (H x W x D)	mm	530 x 900 x 320	530 x 900 x 320
Item code with 30 l		**W3T360169**	**W3T360171**
Item code with 60 l		**W3T360174**	**W3T360176**

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Protocol

1. PCR Reaction: Thaw and assemble the following components in a 0.2 ml PCR tube on ice just prior to use:

   COVID-19 Primers, COVID-19 Probes, 2X RT-qPCR Master Mix, and RT-qPCR Enzyme Mix. Caution: Do not add more than one RNA sample into a single qPCR tube. Mix gently. If necessary, centrifuge briefly.

   Use the Nuclease-free H2O for the Negative Control while using Positive Control Template for the Positive Control setup. Cap tubes and place in the thermal cycler.

Component	20 µl Patient Sample	20 µl Positive Extraction Control	20 µl Negative Extraction Control	Negative Control
RNA Sample	5 µl	0 µl	0 µl	0 µl
COVID-19 Primers	1 µl	1 µl	1 µl	1 µl
COVID-19 Probes	1 µl	1 µl	1 µl	1 µl
2X RT-qPCR MasterMix	10 µl	10 µl	10 µl	10 µl
RT-qPCR Enzyme Mix	0.4 µl	0.4 µl	0.4 µl	0.4 µl
Positive Extraction Control	0 µl	5 µl	0 µl	0 µl
Negative Extraction Control	0 µl	0 µl	5 µl	0 µl
Nuclease – Free H_2O	2.6 µl	2.6 µl	2.6 µl	7.6 µl

2. Use the Nuclease-free H2O for the Negative Control while using Positive Control Template for the Positive Control setup. Cap tubes and place in the thermal cycler. Liquid handling recommendation using 2-125µl or 5-250µl pipette.

VistaLab™ TECHNOLOGIES

8-channel (E8)
2-125µl self standing
Electronic pipette

8-channel (E8)
5-250µl self standing
Electronic pipette

3. Process in the thermal cycler for 42 cycles as follows:

Steps	Temperature/Time	Cycle
cDNA Synthesis	15 minutes at 42°C	1
Pre-Denaturation	10 minutes at 95°C	1
Denaturation	15 seconds at 95°C	40
Annealing	60 seconds at 60°C	
Melting curve	Refer to specific guidelines for instrument used	

Note:
Optimal conditions for amplification will vary depending on the primers and thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.

4. Detection: As three channels (FAM, ROX, HEX) are used in this one tube qPCR assay, we recommend to perform the channel calibration as requested by its manufacturer. Please refer to the instrument's user manual to perform this calibration. Choose the FAM, ROX, and HEX channels for each sample to be tested with the LifeDireX COVID-19 RT-qPCR Detection Kit. Select "None" for ROX passive reference on any qPCR machine requiring ROX as the reference dye.

Expected Performance of Controls

Control Type	Used to Monitor	Expected Results and Ct Values		
		N (FAM)	RP (HEX)	RdRP (ROX)
Positive	Flawed assay setup and reagent failure, including degraded primer and probe	Positive Ct < 40.0	Negative Ct ND	Positive Ct < 40.0
Positive Extraction Control ("RP")	Poor specimen lysis, undesirable specimen collection, improper assay setup, extraction failure, or PCR inhibition	Negative Ct ND	Positive Ct < 40.0	Negative Ct ND
Negative ("NTC")	Assay or extraction reagent contamination	Negative Ct ND	Negative Ct ND	Negative Ct ND
Negative Extraction Control	Cross-contamination	Negative Ct ND	Positive Ct < 40.0	Negative Ct ND

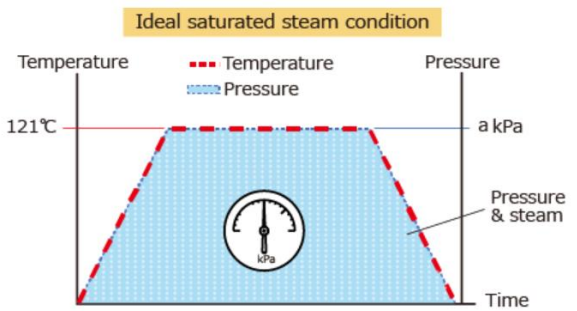
ND = Not Detected. Results are considered invalid if any control does not perform as specified above.

Steps for RNA Isolation 7

Interpretation of Results

SARS-CoV-2			Interpretation	Action
N	RdRP	RP		
+	+	+/-	Positive	Report result to sender health authority.
If only one of the two targets is positive.		+/-	Inconclusive Result	Repeat RT-qPCR of samples or repeat from extraction step. If result is still inconclusive, recommend collection of new specimen(s) from the patient.
-	-	+	Negative	SARS-CoV-2 not detected. Report result to sender health authority
-	-	-	Invalid Result	Repeat from extraction step. If the repeated result remains invalid, recommend collection of a new specimen(s) from the patient.

After experiment, place all the coronavirus-related medical waste has to be autoclaved at 121 ˚C 15 PSI for 30 minutes . Recommend TOMY FLS-1000, low profile large capacity autoclave.




ACA-1000B


ACA-1000K


ACA-1000TP

Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when quantify of nucleic acid targets with the kit.

Trouble	Cause	Solution
Poor Signal or No Signal	Inhibitor Present	1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. 2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Degraded Template Material	1. Do not store diluted template in water or at low concentrations. 2. Check the integrity of template material by automated or manual gel electrophoresis.
	Inadequate Thermal Cycling Conditions	1. Try using a minimum extension time of 30 sec for genomic DNA and 15 sec for cDNA.
Signal in Negative Control	Contamination of Reaction Components with Target Sequence	1. To minimize the possibility of contamination of PCR components by PCR product or other template, designate a work area exclusively for PCR assay setup. 2. Use a solution of 10% bleach instead of ethanol to prepare the workstation area for PCR assay setup. Ethanol will only induce precipitation of DNA in your work area, while the 10% bleach solution will hydrolyze, as well as dissolve, any residual DNA.
Poor Reproducibility Across Replicate Samples	Inhibitor Present	1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. 2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Primer Design	1. Verify primers design at different annealing temperatures.
Low or High Reaction Efficiency	Primer- Dimer	1. Reduce primer concentration. 2. Evaluate primer sequences for complementarity and secondary structure. Redesign primers if necessary. 3. Perform melt-curve analysis to determine if primer- dimers are present.
	Insufficient Optimization	1. Use a thermal gradient to identify the optimal thermal cycling conditions for a specific primer set.

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