CHAI

Coronavirus Environmental Test Kit

Product Manual

RT-qPCR assay for the detection of SARS-CoV-2

Catalog #T1401
96 tests/kit

For Research Use Only

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1. INTRODUCTION

1.1 BACKGROUND

The acute respiratory disease known COVID-19, caused by a novel coronavirus (SARS-CoV-2), was officially declared a pandemic by the World Health Organization (WHO) on March 11, 2020. With widespread test kit and equipment shortages, the spread of COVID-19 has raised international concerns on how to address the public health emergency and prevent the continued spread of the virus.

Environmental testing of public spaces provides a proactive alternative to human laboratory testing to monitor and prevent the spread of highly contagious and easily transmittable diseases. By testing high-contact environmental surfaces such as door handles, tables, PIN pads, bathroom stalls, grocery carts, phones, etc, facilities can monitor the presence of coronavirus and take additional action as necessary such as increased hygiene protocols, recommending diagnostic testing for workers, or surgical closures.

1.2 SCOPE OF WORK

The Coronavirus Environmental Test Kit is designed as a turnkey solution for environmental coronavirus (SARS-CoV-2) monitoring. The intended use of this kit is for research use only (RUO). Neither the kit nor the results may be used to diagnose human patients or clinical samples (i.e nasopharyngeal samples). The kit is intended for environmental swab sampling of hard surfaces and common areas such as tables, door handles, PIN pads, etc. Chai does not support nor validate results for purposes outside of its intended use.

1.3 PRINCIPLE

This kit utilizes probe-based multiplex RT-qPCR on environmental surface samples to simultaneously detect RNA from the SARS-CoV-2 virus and an Internal Control. The kit relies on the available published data from the SARS-CoV-2 genome and targets the N gene of the virus. No cross reactivity with any other coronavirus family members that cause SARS-related diseases (i.e MERS, SARS) has been found. The Internal Control is a synthetic single stranded DNA oligonucleotide which shows no cross reactivity with SARS-CoV-2 and is used to detect PCR inhibition and prevent false negatives.

1.4 VALIDATION

The Coronavirus Environmental Test Kit is validated for use on plastic, stainless steel, brass, rubber, and ceramic surfaces. The limit of detection (LoD) of 290 copies/swab was determined by inoculating an environmental swab with heat-inactivated SARS-COV-2 viral particles. A full validation report including an analytical sensitivity, specificity, and limit of detection is available on the Chai website.

The test kit has been validated on Chai's dual channel Open qPCR instrument and is expected to be compatible with other Real-Time PCR instruments capable of detecting the FAM and HEX fluorophores.

1.5 KIT COMPONENTS AND STORAGE

The kit provides all necessary materials and reagents to run 96 reactions including positive and negative controls. Store all reagents according to specifications.

Component	Quantity	Storage
Dry environmental transport swabs, sterile	96 swabs	Room temperature (25 ˚C)
One-Step DNA/RNA Extraction Buffer	20 mL	Room temperature (25 ˚C)
DNase/RNase - Free Water	5 x 5 mL	Room temperature (25 ˚C)
DNase/RNase - Free Water	0.5 mL	Room temperature (25 ˚C)
PCR Tube and Cap Strips	15 x 8 well strips	Room temperature (25 ˚C)
Coronavirus Environmental Oligo Mix (primers, probes, Internal Control)	475 µL	-20 ˚C
Sahara One-Step RT-qPCR Master Mix with UNG	1.1 mL	-20 ˚C
Cofactor Buffer	65 µL	-20 ˚C
SARS-CoV-2 N Positive Control	85 µL	-20 ˚C

1.4 ADDITIONAL REQUIRED MATERIALS

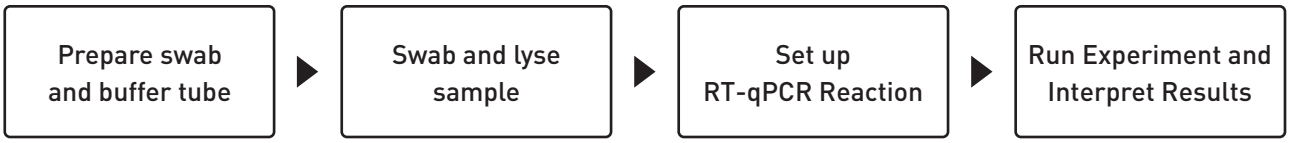
The following lab equipment and consumables are required.

Required Material	Suggested Model
Real-Time PCR Thermocycler (with FAM and HEX fluorophore detection)	Open qPCR Dual Channel, Catalog # E013201 Also available as part of Coronavirus Environmental Starter Pack, Catalog # B2001S
Mini benchtop centrifuge (minimum speed of 5,000 g / approx. 10k RPM)	Mini Centrifuge 10k RPM with Combined Rotor, Catalog # E050101 Also available as part of Coronavirus Environmental Starter Pack, Catalog # B2001S
Pipettes and filtered sterile pipette tips (2 – 20 µL and 20 – 200 µL range)	Available as part of Coronavirus Environmental Starter Pack, Catalog # B2001S
PCR tube rack	
1.5 mL tube rack	
1.5 mL microcentrifuge tubes	

2. WORKFLOW

2.1 OVERVIEW

Figure 1. Kit workflow from environmental swabbing to post-qPCR analysis



2.2 PREPARATIONS

1. The use of PPE (personal protective equipment) is essential during sample collection and the qPCR setup to minimize the risk of exposure to infectious viruses or unknown bacteria. It is always recommended to use disposable gloves and wear eye protection gear, a face mask, and lab coat when performing any part of the kit workflow.

2. The One-Step DNA/RNA Extraction Buffer should be pre aliquoted into 1.5 mL microcentrifuge tubes prior to sample collection in order to maximize workflow efficiency. Prepare an aliquot of 200 µL into a 1.5 mL tube for each sample. For example, if five surface samples are going to be taken, five tubes containing 200 µL of buffer each should be prepared. Users can prepare 1.5 mL tubes with One-Step DNA/RNA Extraction Buffer as needed or pre aliquot all provided One-Step DNA/RNA Extraction Buffer into 1.5 mL tubes at once. Microcentrifuge tube sizes other than 1.5 mL will not be compatible with the swab. Label individual tubes with sample collection names prior to environmental sampling.

3. Swabs must be pre-wetted prior to sample collection to ensure that the bulb of the swab is moist. Unscrew the swab from its individual transport tube and briefly dip the bulb into one of the provided 5 mL bottles of DNase/RNase - Free Water to wet it, then immediately place it back into the transport tube. Swabs that have been pre-wetted will stay moist in the transport tube for up to 24 hours. One 5 mL bottle of DNase/RNase - Free Water may be used to wet multiple swabs. Failure to wet the swab before swabbing may affect the ability of the swab to pick up the virus.

   Note: Swabs are intended for single use only. Do not re-wet any swabs that have been used for environmental swabbing. This will contaminate the DNase/RNase - Free water source.

2.3 SURFACE SWAB COLLECTION

1. To collect an environmental swab sample, pop open and remove the pre-wetted swab from its transport tube at the location of the sample surface. Apply gentle pressure to the moistened swab against the surface and begin swabbing the surface at a 30 – 45 degree angle. Rotate while swabbing to further increase contact with the sample surface area. Cover as much surface area as possible from top to bottom five times and left to right five times for adequate surface area coverage.

2. Immediately place the swab into a labeled 1.5 mL tube containing 200 µL of pre-aliquoted One-Step DNA/RNA Extraction Buffer to lyse the sample. This will extract any RNA from SARS-CoV-2 that was picked up on the sample swab and inactivate the virus in the buffer. **Samples must be lysed immediately after swab collection to limit the risk of spreading active viruses or other live microorganisms.** Ensure that the bulb of the swab is completely submerged in the buffer. Once submerged, use the plastic shaft to

rotate the swab at least 10 times to release the sample cells into the extraction buffer. Then, press the bulb up against the side of the tube while rotating upwards to release excess buffer from the swab.

3. Remove the swab from the tube and put it back in the original transport tube, then cap the 1.5 mL tube. Note that some of the One-Step DNA/RNA Extraction Buffer will be absorbed naturally by the swab. Cap the swab transport tube completely to prevent contamination of any remaining active viruses or microorganisms. The capped swab tube may be disposed of per local regulatory EHS guidelines.

2.4 STORAGE AND TRANSIT OF LYSATE

The 1.5 mL tube now contains the sample lysate required for RT-qPCR. The lysate can be stored for up to 72 hours at room temperature, 4 °C or -20 °C. For longer storage, store the lysate at -80 °C. Failure to adhere to the storage recommendations may affect the sensitivity of the assay and cause false negatives in downstream analysis.

2.5 RT-qPCR REACTION SETUP

1. Ensure that all RT-qPCR reaction setup is in an area with still air (no active air flow) to prevent potential contamination. Thaw the Sahara One-Step RT-qPCR Master Mix with UNG, Coronavirus Environmental Oligo Mix, Cofactor Buffer, and Positive Control at ambient temperature completely. Also thaw sample lysate tubes if frozen at -20 °C or -80 °C. Mix the contents of all samples and reagent tubes by inverting, flicking the tubes, or briefly vortexing for 1 - 3 seconds.

2. Spin all tubes down using a mini centrifuge at a minimum of 5000 rpm for 15 seconds to collect the solution at the bottom of the tubes. Set aside the Positive Control tube and 0.5 mL tube of DNase/RNase - Free Water to be used later for the Positive Control and No Template Control (NTC), respectively.

3. Determine the number of PCR reactions required for the run. Count the Positive Control, No Template Control and each lysate sample as an individual reaction. If two samples are run, for example, four reactions will be required: one for the Positive Control, one for NTC, and two for the environmental swab samples.

4. Prepare a Common Mix containing the Sahara One-Step RT-qPCR Master Mix with UNG, Coronavirus Environmental Oligo Mix, and Cofactor Buffer for the number of PCR reactions. Do not add the control solution or sample lysate to the Common Mix. Multiply the volume required for the Sahara One-Step RT-qPCR Master Mix with UNG, Coronavirus Environmental Oligo Mix, and Cofactor Buffer by the total number of reactions. Include an excess 10% in the calculation to account for pipetting error. **Table 1** shows the amount required for a single reaction. The calculations for each are as follows, where N is the number of total PCR reactions. **Table 2** shows the Common Mix component required for 1 - 16 reactions.

   Volume of Sahara One-Step RT-qPCR Master Mix with UNG (µL) = 11 x N
   Volume of Coronavirus Environmental Oligo Mix (µL) = 4.84 x N
   Volume of Cofactor Buffer (µL) = 0.66 x N

Table 1. Common Mix for a single reaction set up

Component	Volume (µL) per Reaction	Volume (µL) per Reaction (including excess 10%)
Sahara One-Step RT-qPCR Master Mix with UNG	10.00	11.00
Coronavirus Environmental Oligo Mix	4.40	4.84
Cofactor Buffer	0.60	0.66
Total Common Mix	15.00	16.50

Table 2. Amount of each Common Mix component to pipette, 1 – 16 total reactions

Component	1	2	3	4	5	6	7	8
Sahara One-Step RT-qPCR Master Mix with UNG	11.00	22.00	33.00	44.00	55.00	66.00	77.00	88.00
Coronavirus Environmental Oligo Mix	4.84	9.68	14.52	19.36	24.20	29.04	33.88	38.72
Cofactor Buffer	0.66	1.32	1.98	2.64	3.30	3.96	4.62	5.28
Total Common Mix	16.5	33.00	49.50	66.00	82.50	99.00	115.50	132.00

Component	9	10	11	12	13	14	15	16
Sahara One-Step RT-qPCR Master Mix with UNG	99.00	110.00	121.00	132.00	143.00	154.00	165.00	176.00
Coronavirus Environmental Oligo Mix	43.56	48.40	53.24	58.08	62.92	67.76	72.60	77.44
Cofactor Buffer	5.94	6.60	7.26	7.92	8.58	9.24	9.90	10.56
Total Common Mix	148.50	165.00	181.50	198.00	214.50	231.00	247.50	264.00

5. Pipette the required volume of each component of the Common Mix into one labeled 1.5 mL tube. Briefly vortex or pipette up and down to mix, then spin the tube down in a balanced centrifuge for 15 seconds to collect the solution at the bottom of the tube.

6. Using a sterile razor or pair of scissors, cut down the number of PCR tubes and caps required for all reactions. Follow Step 6A or 6B depending on the model of thermocycler used.

   A. If using the Open qPCR thermocycler, cut down the number of provided PCR Tube and Cap Strips required for all reactions. Place the strip of tubes in a PCR tube rack and proceed to Step 7.

   B. If using a thermocycler that utilizes different PCR tubes, use alternate compatible consumables and proceed to Step 7.

7. Aliquot 15 µL of the common mix into each of the PCR tubes.

8. Assign labels to the individual PCR tubes from left to right starting with the Positive Control and NTC. Document the layout in a laboratory notebook to keep track of the corresponding well for each control or sample.

   Label the side starting with the Positive Control with a dot to keep track of the strip layout; do not write on the top or side of the tubes or caps themselves to avoid interference with the optical readout of the thermocycler.

   Add the required template (Positive Control, NTC, or environmental sample) to each well as indicated below in **Table 3**. Add the environmental samples first, then add the NTC. Add the Positive Control last to minimize potential contamination and immediately cap the tube after pipetting.

   See **Figure 2** for a sample PCR strip layout.

   Table 3. Template to add for each Well Type

Well Type	Template to Add
Positive Control	5 µL of Positive Control
No Template Control (NTC)	5 µL of DNAse/RNAse - Free Water (Use the 0.5 mL tube provided)
Environmental Sample	5 µL of the sample lysate

   Figure 2. Example for the PCR strip layout in a 16-well thermocycler

	1	2	3	4	5	6	7	8
A	Positive Control	NTC	Gas Pump #1	Gas Pump #3	Table #1	Table #3	Door Knob #1	Front Door Handle #1
B	PIN Pad #1	PIN Pad #2	Gas Pump #2	Gas Pump #4	Table #2	Table #4	Door Knob #2	Front Door Handle #2

9. Cap the PCR tubes completely and spin down the strips with a mini centrifuge for 2 minutes at a minimum of 5,000 g (10k rpm on most mini centrifuges). Ensure that no air bubbles or solution remains on the sides or top of the tubes.

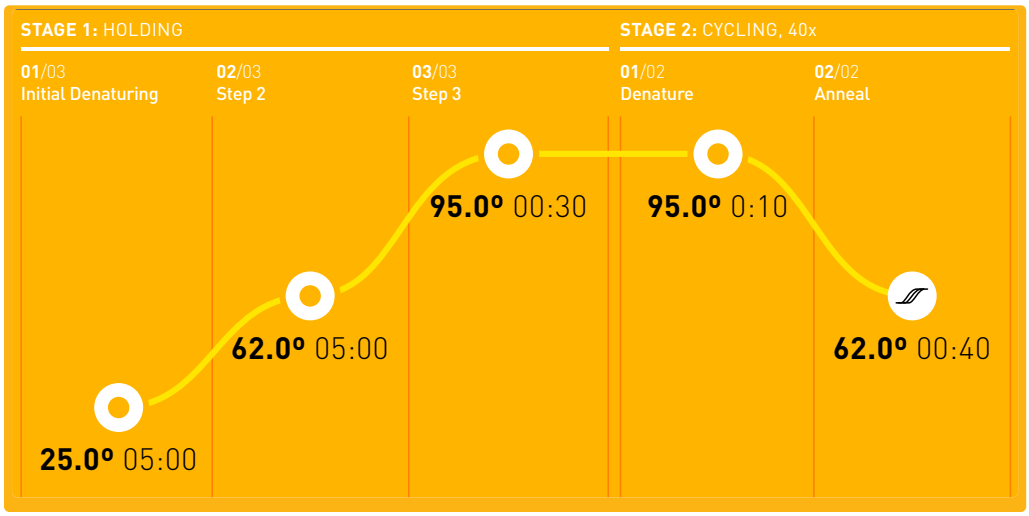
10. Load the PCR strips into the thermocycler.

11. Using the thermocycler software, label the wells with the names of all samples and set the software with the following cycling protocol (**Table 4**). The data collection point should be set at the final 62 °C annealing step. If required by the software, assign the SARS-CoV-2 target as Channel 1 (FAM) and the Internal Control target as Channel 2 (HEX) and label controls and samples as necessary. For the PCR protocol using the dual channel Open qPCR, consult **Figure 3**. Start the PCR run.

Table 4. PCR protocol

Temperature	Time	# Cycles
25 ºC	5 min	1
62 ºC	5 min	
95 ºC	30 sec	
95 ºC	10 sec	40
62 ºC	40 sec	

Figure 3: PCR protocol in Open qPCR



3 POST RUN ANALYSIS

3.1 INTERPRETING RESULTS

Before concluding that a sample is positive or if the presence of coronavirus is not detected, it is essential to assess the validity of controls and samples to ensure that successful PCR has taken place based on the presence or absence of the Channel 1 and Channel 2 targets. The presence of the SARS-CoV-2 target is detected in Channel 1 (FAM), while Channel 2 (HEX) is used for the Internal Control. The Internal Control is used for detecting PCR inhibition and preventing false negatives. For adequate quality control, one Positive Control and one No Template Control (NTC) should be processed with each run.

Table 5. Control results evaluation

Positive Control (Ch1 - FAM)	Positive Control (Ch2 - HEX)	NTC (Ch1 - FAM)	NTC (Ch2 - HEX)	Result
POS	POS	NEG	POS	Positive Control and NTC are both Valid
	NEG			
NEG	POS	NEG	POS	Positive Control is Invalid, NTC is Valid
	NEG			
POS	POS	POS	POS	Positive Control is Valid, NTC is Invalid
	NEG		NEG	
POS	POS	NEG	NEG	Positive Control is Valid, NTC is Invalid
	NEG			

If the Positive Control and NTC are found to be valid, then samples can be evaluated for the presence of SARS-CoV-2. Amplification in Channel 1 indicates that SARS-CoV-2 was detected in the environmental sample. SARS-CoV-2 was not detected in the environmental sample if there is no amplification in Channel 1.

Table 6. Sample results evaluation

Channel 1 SARS-CoV-2 Target	Channel 2 Internal Control Target	Result
POS	POS	SARS-CoV-2 Detected
	NEG	
NEG	POS	SARS-CoV-2 Not Detected
NEG	NEG	Invalid Sample: Repeat the test

3.2 EXPORTING RESULTS

If using the dual channel Open qPCR, results can be exported in CSV format for future analysis. From the Amplification Curve results page, select "Export" to download a .zip file containing information on the individual run including amplification data, temperature logs, and C_q values.

3.3 TROUBLESHOOTING

PROBLEM	POSSIBLE CAUSE	SOLUTION
No fluorescence signal detected in any samples from both channels, including positive and negative control	Incorrect concentration of Common Mix components	Ensure that the correct volume of each reagent is aliquoted during preparation of the Common Mix
	Omitted Common Mix components	Verify that all components (Sahara One-Step RT-qPCR Master Mix with UNG, Coronavirus Environmental Oligo Mix and Cofactor Buffer) are added to the Common Mix
	Incorrect PCR protocol	Utilize the correct cycling protocol for the RT-qPCR run as specified above.
No Positive Control amplification in the FAM channel	No Positive Control added to PCR tube	Add 5 uL of positive control to the correct assigned well
No amplification from the Internal Control for an environmental sample	PCR inhibition	Avoid picking up excess dirt or debris with the swab bulb when surface swabbing to avoid excessive PCR inhibition
		Dilute the lysate in a 1:10 ratio using the One-Step DNA/RNA Extraction Buffer by adding 1.08 mL to the tube containing the sample lysate, then re-run the sample. Add 5 µL of the diluted lysate into the PCR tube as usual.
Irregular baseline of the amplification curves	Pipetting error	Practice proper pipetting technique to avoid bubbles in the PCR reaction wells
	Air bubbles in wells / PCR strips not spun down	Spin down the PCR strips for 2 minutes and check that the wells do not have air bubbles prior to the PCR run. Air bubbles and solution on the sides or top of the PCR tubes will interfere with the fluorescence measurements.
	Unstable thermocycler location	Make sure the thermocycler is placed in a secure location that is free of vibrations resulting from other nearby equipment

PROBLEM	POSSIBLE CAUSE	SOLUTION
Low RFU plateau of amplification curves	PCR inhibition	Inhibition may be occurring in the sample. Dilute the lysate sample by 1:10 using One-Step DNA/RNA Extraction Buffer and re-run the sample
	Pipetting error	Ensure that no solution is removed after being pipetted into a PCR strip well to avoid altering the primer and probe concentrations of each reaction. All the wells should have an equal volume of 20 µL.
Amplification in NTC reaction	Carry-over contamination	Take measures to avoid potential sources of contamination. Change pipettes tips between samples, clean pipettes, use filtered tips, etc.
	Contamination of the extraction/preparation area	Clean surfaces and instruments with aqueous detergents, wash lab coats, and replace test tubes and tips in use. Ensure that all sample prep is performed in a still air environment.

3.4 LIMITATIONS

1. Environmental samples must be collected, transported, and stored using the recommended procedures as described above. Improper collection, transport, or storage may limit results.
2. Adhere to the manual guidelines for reliable results. Changes in reaction setup or cycling protocol may lead to experiment failures or inconsistencies.
3. Spontaneous mutations within the target sequence can affect the detection of the target sequence. The performance of Coronavirus Environmental Kit has been validated using all the publicly available nucleic acid sequences for SARS-CoV-2 as of May 1, 2020.
4. Negative environmental testing results cannot exclude the presence of SARS-CoV-2 within the setting where the investigation has been conducted.
5. Results should be interpreted after verifying the validity of all controls and samples.. Interpretation must be performed by trained personnel.
6. Negative results indicate that the amount of SARS-CoV-2 present in the testing area is lower than the limit of detection but do not guarantee the complete absence of the virus.

4 ADDITIONAL SUPPORT

4.1 RELATED DOCUMENTATION

Document	Document Number
Coronavirus Environmental Test Kit Validation Report	002280
Coronavirus Environmental Test Kit Safety Data Sheet	002293
Open qPCR User Manual	001892
One-Step DNA/RNA Extraction Buffer Manual	002243

4.2 REFERENCES

World Health Organization. (2020). Coronavirus disease (COVID-19) outbreak: rights, roles and responsibilities of health workers, including key considerations for occupational safety and health: interim guidance, 19 March 2020. World Health Organization.

World Health Organization. (2020). Surface sampling of coronavirus disease (COVID-19): a practical "how to" protocol for health care and public health professionals, 18 February 2020, version 1.1. World Health Organization.

4.3 TRADEMARKS

4.4 CONTACT

Additional documentation and information about this kit can be found at www.chaibio.com/coronvirus.
For technical assistance, contact support@chaibio.com or a local distributor.

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