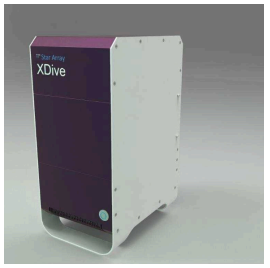
Nov 09, 2020 Version 2

OnsiteGene 4 Protocol Saliva Extract V.2



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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Abstract

This OnsiteGene protocol is designed for testing the normal saliva samples with nucleic acid extraction. A normal saliva collection tube is used for the collection. The protocol uses the Star Array® Hi-Sense™ COVID-19 Molecular Testing Kit 1.0 in the one-step real-time RT-qPCR test assay to qualitatively detect RNA from SARS-CoV-2 virus in the human respiratory specimen. It combines the reverse transcription technology and real-time PCR method to provide accurate detection of the SARS-CoV-2 coronavirus. The Star Array® magnetic beads RNA extraction instrument and kit can automatically extract and purify the RNA for 32 samples in 6 minutes. It is ideal for the saliva samples that contain high amount of PCR inhibitors and can't be processed by the direct amplification. The triplex fluorescence design of the kit simultaneously detects the N1 and Orf1ab genes of the virus and the human RNase P gene as an internal control to ensure the sample quality. This protocol uses the Star Array® XDive™ Superfast Real-Time PCR instrument to perform 40-cycle PCR in 8 minutes, and can test up to 16 samples or controls in each run. The total protocol time from sample collection to data interpretation is less than 15 minutes.

The sample collection steps is adapted from the published protocol:
https://www.protocols.io/view/saliva-collection-and-rna-extraction-for-sars-cov-bh6mj9c6

Materials

Instruments and Consumables

	Instrument and Consumable	Supplier	Catalog #
	Real-Time PCR	OnsiteGene	XDIVE-16
	RNA extraction instrument	OnsiteGene	IS3000001
	Mini centrifuge	Heathrow Scientific	HS120395
	Vortex	Four E's Scientific	4ESS-MI0101002D
	Reaction tube and cap	OnsiteGene	CM1010001
	Pipette set	Microlit	RBOKit
	Pipette tips	Extragene	TS-10-RS

Sample Colletion and Extraction Kit

	Reagent or Consumable	Supplier	Catalog #
	1.5ml microcentrifuge tube	Biologix	88-915X
	Purifier extraction kit	OnsiteGene	KT3000001
	Purifier cartridge	OnsiteGene	CM3000001
	Storage solution	OnsiteGene	RG000001

PCR Reaction Reagents

	Reagent	Supplier	Catalog #
	Hi-Sense COVID detection kit	OnsiteGene	KT1010001
	External positive control	ZeptoMetrix	NATSARS(COV2)-ERC

Primer and Probe Sequences

	Target Number	Target Gene	Sequence/antibody
	1_F	N1	GACCCCAAAATCAGCGAAAT
	1_R	N1	TCTGGTTACTGCCAGTTGAATCTG
	1_P	N1	ACCCCGCATTACGTTTGGTGGACC
	2_F	ORF1ab	TATGTGGAAAGGTTATGGCTGTAG
	2_R	ORF1ab	GATTGTGCATCAGCTGACTGAAG
	2_P	ORF1ab	TTGTGATCAACTCCGCGAACCCATG
	3_F	Rnase P	AGATTTGGACCTGCGAGCG
	3_R	Rnase P	GAGCGGCTGTCTCCACAAGT
	3_P	Rnase P	TTCTGACCTGAAGGCTCTGCGCG

Safety warnings

Most institutions will require samples potentially containing full-length SARS-CoV-2 RNA to be handled in a biosafety level 2 cabinet. Please seek guidance from your local biosafety office on specific recommendations for working with samples which could contain live SARS-CoV-2 virus.

Sample Collection Procedure

1m

1 Saliva sample should be collected with the assistance of a healthcare worker or technician.

2 Before collection, clean hands using alcohol-based sanitizer or soap and water (no fragrances) and wear appropriate PPE (at minimum, gloves and a mask).

2.1 Back away as far as possible from the sample donor during the whole process. While preparing collection materials, direct the sample donor to begin pooling saliva in their mouth.

2.2 This protocol is intended for the collection of the normal saliva that naturally pools into the mouth. No coughing or sniffing prior to sample collection is required. Ideally, water should be avoided 10 minutes prior to collection. Other drinks, food, and nasal sprays should be avoided half an hour before sample collection.

3 Ensure all collection materials are labelled with the correct identifying information.

4 Open the lid of the saliva collection tube, and direct the sample provider to gently expel saliva into the container until at least 500 µL is collected. Securely replace the lid of the collection tube.

1m

4.1 The total volume measured should exclude any bubbles.

5 Add 500 µL of OnsiteGene storage media to each sample tube and secure the lid of each tube.

5.1 Sterilize the tube surface with 70% ethanol or a disinfecting wipe, and place the sample in a secondary container or an appropriately labeled biohazard bag.

5.2 Dispose of gloves, and register the sample collection information (including date and time).

6 Transfer the sample at room temperature for sample processing. The virus RNA remains stable at room temperature for 3-5 days.

Real-Time PCR Procedure

9m 45s

7 Vortex each sample collection tube for 5 seconds at 3000-5000 RPM. 5s

7.1 This step can be substituted by tapping or shaking each sample collection tube to mix the sample.

8 Spin down the tube for 15 seconds using centrifuge. 15s

8.1 The purpose is to: 1) Remove liquid from the cap to avoid the contamination when cap is removed; 2) Spin down solid particles to the bottom of the tube and allow supernatant to be acquired.

9 Transfer 900 µL supernatant of the sample into the cartridge of magnetic beads RNA extraction instrument from OnsiteGene. 5s

10 Load the cartridge into the extraction instrument to extract and purify the viral RNA. The duration of the process is 6 minutes without any hand-on work. The sample elution volume is 50 µL after the extraction. The extraction instrument can process 32 samples in one run. 6m

11 Transfer 7.5 µL extracted RNA sample (this sample can be stored at -20°C for 14 days) from the cartridge and 7.5 µL OnsiteGene Hi-Sense^{TM} COVID-19 detection kit reaction mix (with primers and probes) into a superfast RT-qPCR reaction tube. Secure the lid of the tube. 5s

12 Spin down the tube for 15 seconds using centrifuge or spinner. 15s

12.1 This step can be substituted by hand-shaking the tube to get the samples to the bottom.

13 Load up to 16 superfast RT-qPCR reaction tubes onto the OnsiteGene XDive Superfast Real-Time PCT instrument and run the following conditions: 8m

	Step	Temperature	Time
	1	50°C	30 sec
	2	95°C	0 sec
	3	60°C	2 sec

	4	Read Fluorescence intensity and overhead	(10 sec)
	5	Repeat steps 2-4 for 40 cycles.	

a) XDive takes 8 minutes to complete 40 thermal cycles with fluorescence imaging.
b) Run external positive control and external negative control twice per day using 5 µL of positive control and no-template control (NTC - water). The control tests are recommended to be performed at the beginning and the end of the work/day.

14 15.Report results per the following interpretation criteria:

	Results	**IC CT**	**SARS-Cov-2 Targets**		**Interpretation**
			ORF1ab CT	**N1 CT**	
	Negative	< 35	≥ 40	≥ 40	Indicate the absence of SARS-Cov-2 RNA
	Positive	any value	< 40	any value *	Indicate the presence of SARS-Cov-2 RNA
			any value *	≤ 40	
	Invalid	≥ 35	≥ 40	≥ 40	Inability to conclusively determine presence or abse

					nce of SARS-Cov-2 RNA. This may be due to 1) Internal Control failure; or 2) failure to detect sufficient specimen volume. The sample needs to be retested.

* In the case of one SARS-Cov-2 target positive (CT < 40) and one SARS-Cov-2 target negative (CT ≥ 40), the result is suggestive of: 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in one of the target regions, or 3) other factors.