Saliva Collection and RNA amplification

Reagent	Source	Identifier
QuickExtract DNA Extraction Solution	Lucigen	QE09050
NASBA liquid kit	Life Sciences Advanced Technologies Inc.	SKU: NWK-1
Tris (1 M), pH = 8, RNase free	Invitrogen	AM9855G
Sodium Hydroxide	Sigma-Aldrich	71687
1M $MgCl_2$	Invitrogen	AM9530G
2M KCl	Invitrogen	AM9640G
DTT	Sigma-Aldrich	43816
DMSO	Sigma-Aldrich	276855
dNTP set, 100 mM	Invitrogen	10297018
NTP set, 100 mM	Thermo Scientific	R0481
Bio-11-UTP (75 mM)	Invitrogen	AM8451
RNase H	NEB	M0297L
ProtoScript II reverse transcriptase	NEB	M0368S
T7 RNA polymerase	NEB	M0251L
BSA 20 mg/ml	NEB	B9000S
Direct-zol RNA Miniprep Plus	Zymo Research	R2070
PCRD lateral flow assay	Abingdon Health	FG-FD51673
Qubit RNA HS Assay Kit	Invitrogen	Q32852
PowerUp™ SYBR™ Green qPCR Master Mix	Applied Biosystems	15340939

1. Lysis of saliva samples Mix crude saliva at 1:1 ratio with QuickExtract DNA Extraction Solution. Incubate at 95 °C for 5 min to ensure complete lysis of virus and inactivation of proteinase K.

2. NASBA reaction Take 1 µl from the product of Step 1 and add into the NASBA reaction mixture to make a total volume of 20 µl. Reaction mixture can either be prepared in-house or from the Life Sciences NASBA liquid kit (see tables below). a. Reaction mixture without the enzyme mix is incubated at 65 °C for 2 min followed by a 10-min incubation at 41 °C. Following that, 5 µl enzyme mix is added into the reaction and incubated at 41 °C for a further of 90-120 min. b. Alternatively, reaction mixture including the enzyme mix is prepared and incubated directly at 41 °C for a total of 90-120 min.

3. At the end of the NASBA reaction, RNA is purified from the end-product using Direct-zol RNA Miniprep kit, and eluted with 30 µl of RNase free water. After purification, 4.2 µl of purified RNA is mixed with 1.8 µl of FAM labelled RNA capture oligo and 84 µl of PCRD extraction buffer (see table below). Take 75 µl of mix to the sample well of a PCRD test cassette. Results will be shown within 10 min.

Life Sciences reaction mixture (RM)

	vol.	**stock conc.**	**conc. in RM**
sample	1 µl		
primers	1 µl	0.5 µM each	25 nM each
water +/- beacon	3 µl		20 nM for beacon
buffer (NECB-24)	6.7 µl		
nucleotide (NECN-24)	3.3 µl		
enzyme mix (NEC-1-24)	5 µl		
total volume	20 µl		

In-house reaction mixture (RM)

	vol.	**stock conc.**	**conc. in RM**
sample	1 µl		
primers	1 µl	0.5 µM each	25 nM each
water +/- beacon	4 µl		20 nM for beacon
buffer with DMSO*	5 µl		
nucleotide mix*	4 µl		
enzyme mix*	5 µl		
total volume	20 µl		

References

1. Wu, Q., Suo, C., Brown, T., Wang, T., Teichmann, S. A., & Bassett, A. R. (2020). INSIGHT: a population scale COVID-19 testing strategy combining point-of-care diagnosis with centralised high-throughput sequencing. Cold Spring Harbor Laboratory. https://doi.org/10.1101/2020.06.01.127019

2. Qin, Z., Peng, R., Baravik, I. K., & Liu, X. (2020). Fighting COVID-19: Integrated Micro- and Nanosystems for Viral Infection Diagnostics. Matter, 3(3), 628–651. https://doi.org/10.1016/j.matt.2020.06.015