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SPOT2 protocol

Huimin Zhao¹, stlane2¹, Guanhua Xun¹

¹University of Illinois at Urbana-Champaign

XPRIIZE Rapid Covid Testing

stlane2

MATERIALS

STEP MATERIALS

⊗	Magnesium Sulfate (MgSO ₄) Solution - 6.0 ml	New England Biolabs Catalog #B1003S
⊗	E gene primer mix	Contributed by users Catalog #N/A
⊗	Non-primer oligos	Contributed by users Catalog #N/A
⊗	Bst 2.0 WarmStart DNA Polymerase - 8,000 units	New England Biolabs Catalog #M0538L
⊗	WarmStart RTx Reverse Transcriptase - 250 rxns	New England Biolabs Catalog #M0380L
⊗	Isothermal Amplification Buffer - 6.0 ml	New England Biolabs Catalog #B0537S
⊗	Nuclease-free Water - 25 ml	New England Biolabs Catalog #B1500S
⊗	Deoxynucleotide Solution Mix - 8 umol of each	New England Biolabs Catalog #N0447S
⊗	N gene primer mix	Contributed by users Catalog #N/A
⊗	Saliva sample	Contributed by users Catalog #N/A
⊗	Manganese(II) chloride tetrahydrate	Merck MilliporeSigma (Sigma-Aldrich) Catalog #M3634
⊗	Reporter probe 1	Contributed by users Catalog #N/A
⊗	Reporter Probe 2	Contributed by users Catalog #N/A
⊗	Non-CRISPR nuclease	Contributed by users Catalog #N/A

OPEN ACCESS

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

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 41792

- 1 Label test kit bag with patient's name and a unique sample number for the test run. Provide a 20 uL microcap and PCR tube and have patient collect a saliva sample and place it into then seal the PCR tube. Label PCR tube with sample number matching the test kit bag.

- 2 Place PCR tubes into floating rack and insert into sous vide water bath at 95C. Incubate for 5 minutes for sample pretreatment.

Equipment	
Thermal Immersion Cooker	NAME
Sous Vide - low cost waterbath	TYPE
Wancle	BRAND
P00252	SKU

95 °C 00:05:00

- 2.1 During incubation, scan barcodes on test kits and enter patient identifying information into the SPOT2 device.

Equipment	
SPOT2 Device	NAME
Data logging fluorometer	TYPE
University of Illinois	BRAND
N/A	SKU

Equipment

USB Laser Barcode Scanner

NAME

Barcode Scanner

TYPE

WoneNice

BRAND

WN3300

SKU

- 3** After completion of pretreatment, use the second provided 20 uL microcap to transfer a small volume of saliva from the PCR tube to the capillary tube, containing SPOT assay mastermix. Place capillary tubes into floating rack and insert into sous vide water bath at 63C for 30 minutes.

63 °C 00:30:00

SPOT assay mastermix:

	Initial concentration	Final concentration	Amount (μL)
Upper compartment			
WarmStart® Bst 2.0	8000 units/mL	160 units/mL	2
WarmStart® RTx	15,000 units/mL	150 units/mL	1
Isothermal amplification buffer	10X	0.5X	8
dNTPs	10 mM	0.7 mM	5.6
MgSO4	100 mM	4 mM	3.2
N gene primer mix	10X	0.25X	2
E gene primer mix	10X	0.25X	2
Saliva samples			5
Non-CRISPR nuclease	5 mg/mL or 55 μM	1.375 uM	2
MnCl2	50 mM	0.5 mM	0.8
Non-primer oligos (total 6 oligos)	100 μM	625 nM	3
Reporter probe 1	100 μM	156.25 nM	0.125
Reporter probe 2	100 μM	312.5 uM	0.25

Nuclease-free water			44.025
Total			80

⊗ Bst 2.0 WarmStart DNA Polymerase - 8,000 units **New England Biolabs Catalog #M0538L**

🧪 2 µL

⊗ WarmStart RTx Reverse Transcriptase - 250 rxns **New England Biolabs Catalog #M0380L**

🧪 1 µL

⊗ Isothermal Amplification Buffer - 6.0 ml **New England Biolabs Catalog #B0537S**

🧪 8 µL

⊗ Deoxynucleotide Solution Mix - 8 umol of each **New England Biolabs Catalog #N0447S**

🧪 5.6 µL [M] 0.7 millimolar (mM)

⊗ Magnesium Sulfate (MgSO₄) Solution - 6.0 ml **New England Biolabs Catalog #B1003S**

🧪 3.2 µL [M] 4 millimolar (mM)

⊗ N gene primer mix **Contributed by users Catalog #N/A**

⊗ E gene primer mix **Contributed by users Catalog #N/A**

⊗ Saliva sample **Contributed by users Catalog #N/A**

⊗ Nuclease-free Water - 25 ml **New England Biolabs Catalog #B1500S**

⊗ Non-CRISPR nuclease **Contributed by users Catalog #N/A**

⊗ Manganese(II) chloride tetrahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M3634**

🧪 0.8 µL [M] 0.5 millimolar (mM)

⊗ Non-primer oligos **Contributed by users Catalog #N/A**

⊗ Reporter probe 1 **Contributed by users Catalog #N/A**

⊗ Reporter Probe 2 **Contributed by users Catalog #N/A**

4 Transfer floating capillary rack to sous vide at 95C for 5 minutes.

🌡 95 °C ⌚ 00:05:00

- 5 Individually transfer capillary tubes to SPOT2 device and press "Run" on the SPOT software. An integrated RFID chip on each capillary will automatically link test result to the patient information entered previously.

Equipment	
SPOT2 Device	NAME
Data logging fluorometer	TYPE
University of Illinois	BRAND
N/A	SKU