Version 2

Apr 27, 2021

SPOT system protocol V.2

Version 1 is forked from SPOT1 assay

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1 Works for me dx.doi.org/10.17504/protocols.io.bujvnun6

SPOT

stlane2

ABSTRACT

The need for rapid, accurate, and scalable testing systems for COVID-19 diagnosis is clear and urgent. Here we report a rapid Scalable and Portable Testing (SPOT) system consisting of a rapid, highly sensitive, and accurate assay and a battery-powered portable device for COVID-19 diagnosis. The SPOT assay comprises a one-pot reverse transcriptase-loop-mediated isothermal amplification (RT-LAMP) followed by PfAgo-based target sequence detection. It is capable of detecting the N gene and E gene in a multiplexed reaction with the limit of detection (LoD) of 0.44 copies/µL and 1.09 copies/µL, respectively, in SARS-CoV-2 virus-spiked saliva samples within 30 min. Moreover, the SPOT system is used to analyze 104 clinical saliva samples and identified 28/30 (93.3% sensitivity) SARS-CoV-2 positive samples (100% sensitivity if LoD is considered) and 73/74 (98.6% specificity) SARS-CoV-2 negative samples. This combination of speed, accuracy, sensitivity, and portability will enable high-volume low-cost access to areas in need of urgent COVID-19 testing capabilities.

DOI

dx.doi.org/10.17504/protocols.io.bujvnun6

PROTOCOL CITATION

Guanhua Xun, Huimin Zhao, stlane2 2021. SPOT system protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bujvnun6 Version created by Guanhua Xun

FORK NOTE

FORK FROM

Forked from SPOT1 assay, stlane2

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OWNERSHIP HISTORY

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49493

MATERIALS TEXT

STEP MATERIALS

Nuclease-free Water - 25 ml **New England Biolabs Catalog #B1500S** Step 2

Bst 2.0 WarmStart DNA Polymerase - 8,000 units **New England Biolabs Catalog #M0538L** Step 2

Deoxynucleotide Solution Mix - 8 umol of each **New England Biolabs Catalog #N0447S** Step 2

Non-CRISPR nuclease **Contributed by users Catalog #N/A** Step 2

Non-primer oligos **Contributed by users Catalog #N/A** Step 2

E gene primer mix **Contributed by users Catalog #N/A** Step 2

Isothermal Amplification Buffer - 6.0 ml **New England Biolabs Catalog #B0537S** Step 2

N gene primer mix **Contributed by users Catalog #N/A** Step 2

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

WarmStart RTx Reverse Transcriptase - 250 rxns **New England Biolabs Catalog #M0380L** Step 2

Manganese(II) chloride tetrahydrate **Sigma Aldrich Catalog #M3634** Step 2

Magnesium Sulfate (MgSO4) Solution - 6.0 ml **New England Biolabs Catalog #B1003S** Step 2

Reporter probe 1 **Contributed by users Catalog #N/A** Step 2

Saliva sample **Contributed by users Catalog #N/A** Step 2

QuickExtract DNA Extraction Solution **Lucigen Catalog #QE09050** Step 1

1 Using the first provided microcap, collect a saliva sample into capillary 1, containing QuickExtract DNA Extraction Solution (Lucigen). Insert the capillary into the SPOT device and press the "Start" button to run the 5-minute pretreatment.

QuickExtract DNA Extraction Solution **Lucigen Catalog #QE09050**

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SPOT1 Device
Incubating fluorometer
University of Illinois N/A

95 °C

00:05:00

2 After pretreatment, remove capillary 1 from the SPOT device and use the second provided microcap to transfer a small volume of pretreated sample to capillary 2, which contains the SPOT assay mastermix. Dispense the pretreated sample into **only the top layer of the capillary**, as shown in the diagram below. **Disturbing the wax dividing layer during sample transfer may lead to a failed reaction.**

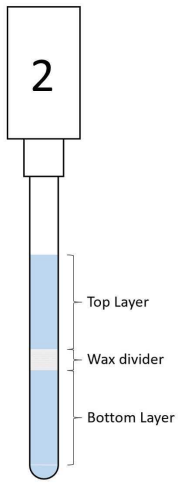

Diagram of capillary 2 layout. Pretreated saliva samples should be transferred into the top layer **only.** Disturbing the wax divider will result in a failed detection reaction.

SPOT assay master mix:

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A	B	C	D
	Initial concentration	**Final concentration**	**Amount (µL)**
Upper compartment			
WarmStart® Bst 2.0	8000 units/mL	320 units/mL	1.6
WarmStart® RTx	15,000 units/mL	300 units/mL	0.8
Isothermal amplification buffer	10X	0.5X	4
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
Nuclease-free water			15.8
Total			40
Lower compartment			
Isothermal amplification buffer	10X	1X	4
PfAgo	5 mg/mL or 55 µM	1.375 uM	2
MnCl2	50 mM	0.5 mM	0.8
gDNAs (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			29.825
Total			40

☒ 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 Milimolar (mM)

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

3.2 µl [M]4 Milimolar (mM)

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

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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 **Sigma Aldrich Catalog #M3634**

0.8 µl **0.5 Milimolar (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**

3 Insert capillary 2 into the SPOT device and press the "Start" button to initiate the 35-minute detection reaction. 35m

SPOT1 device
Incubating fluorometer
University of Illinois **N/A**

63 °C **00:30:00**

95 °C **00:05:00**

4 Result ("Positive"/"Negative"/"Inconclusive") will be displayed on SPOT device LCD screen after completion of detection reaction and the 1-minute cooling period. 1m

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