SEP 08, 2020

SPOT1 assay

Guanhua Xun¹, Huimin Zhao¹, stlane2¹

¹University of Illinois at Urbana-Champaign

XPRIIZE Rapid Covid Testing

SPOT

stlane2

MATERIALS

STEP MATERIALS

✕	Nuclease-free Water - 25 ml New England Biolabs Catalog #B1500S
✕	Bst 2.0 WarmStart DNA Polymerase - 8,000 units New England Biolabs Catalog #M0538L
✕	Deoxynucleotide Solution Mix - 8 umol of each New England Biolabs Catalog #N0447S
✕	Non-CRISPR nuclease Contributed by users Catalog #N/A
✕	Non-primer oligos Contributed by users Catalog #N/A
✕	E gene primer mix Contributed by users Catalog #N/A
✕	Isothermal Amplification Buffer - 6.0 ml New England Biolabs Catalog #B0537S
✕	N gene primer mix Contributed by users Catalog #N/A
✕	Reporter Probe 2 Contributed by users Catalog #N/A
✕	WarmStart RTx Reverse Transcriptase - 250 rxns New England Biolabs Catalog #M0380L
✕	Manganese(II) chloride tetrahydrate Merck MilliporeSigma (Sigma-Aldrich) Catalog #M3634
✕	Magnesium Sulfate (MgSO4) Solution - 6.0 ml New England Biolabs Catalog #B1003S
✕	Reporter probe 1 Contributed by users Catalog #N/A
✕	Saliva sample Contributed by users Catalog #N/A
✕	QuickExtract DNA Extraction Solution Lucigen Catalog #QE09050

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bk25kyg6

Protocol Citation: Guanhua Xun, Huimin Zhao, stlane2 2020. SPOT1 assay. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bk25kyg6>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol in our workspace and it is working.

Created: Sep 08, 2020

Last Modified: Sep 08, 2020

PROTOCOL integer ID:
 41789

Using the first provided microcan, a sample is collected into canillary A containing QuickExtract

- 1 Using the first provided microcap, a sample is collected into capillary A, containing QuickExtract DNA Extraction Solution (Lucigen). Insert the capillary into the SPOT1 device and press the "Start" button to run the 5-minute pretreatment.

QuickExtract DNA Extraction Solution Lucigen Catalog

#QE09050

Equipment

SPOT1 Device

NAME

Incubating fluorometer

TYPE

University of Illinois

BRAND

N/A

SKU

🌡️ 95 °C

🕒 00:05:00

- 2 After pretreatment, remove capillary A from the SPOT1 device and use the second provided microcap to transfer a small volume of pretreated sample to capillary B, which contains the SPOT assay mastermix. Insert capillary B into the SPOT1 device and press the "Start" button to initiate the 35-minute detection reaction.

Equipment

SPOT1 device

NAME

Incubating fluorometer

TYPE

University of Illinois

BRAND

N/A

SKU

🌡️ 63 °C

🕒 00:30:00

🌡️ 98 °C

🕒 00:05:00

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 μM	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 nM	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 μmol 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

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

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

- 3** Result ("Positive"/"Negative"/"Inconclusive") will be displayed on SPOT1 device LCD screen after completion of detection reaction and the 1-minute cooling period.