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SPOT1 revised protocol

Forked from SPOT1 assay

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

SPOT

stlane2

DOI

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

PROTOCOL CITATION

Guanhua Xun, Huimin Zhao, stlane2 2020. SPOT1 revised protocol. **protocols.io** https://protocols.io/view/spot1-revised-protocol-bqfkmtkw

FORK NOTE

For use with the SPOT at-home device.

FORK FROM

Forked from SPOT1 assay, stlane2

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45260

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 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**

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SPOT1 Device
Incubating fluorometer
University of Illinois N/A

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. 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.**

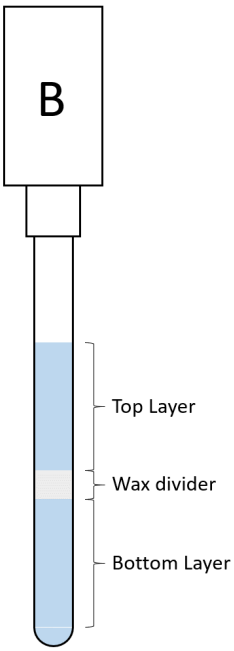

Diagram of capillary B layout. Pretreated saliva samples should be transferred into the top layer **only.** Disturbing the wax divider will result in a failed detection reaction.

SPOT assay master mix:

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	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 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**

☒ 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**

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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 B into the SPOT1 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**

98 °C **00:05:00**

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

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