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SPOT1 assay

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1 Works for me dx.doi.org/10.17504/protocols.io.bk25kyg6

SPOT

stlane2

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PROTOCOL CITATION

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STEPS MATERIALS

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

EQUIPMENT

NAME	CATALOG #	VENDOR

NAME	CATALOG #	VENDOR
SPOT1 device	N/A	
SPOT1 Device	N/A	

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
by Lucigen
Catalog #: QE09050

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. Insert capillary B into the SPOT1 device and press the "Start" button to initiate the 35-minute detection reaction.

SPOT1 device
Incubating fluorometer
University of Illinois N/A

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

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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
by New England Biolabs
Catalog #: M0538L

2 µl

WarmStart RTx Reverse Transcriptase - 250 rxns
by New England Biolabs
Catalog #: M0380L

1 µl

Isothermal Amplification Buffer - 6.0 ml
by New England Biolabs
Catalog #: B0537S

8 µl

Deoxynucleotide Solution Mix - 8 umol of each
by New England Biolabs
Catalog #: N0447S

5.6 µl [M]0.7 Milimolar (mM)

Magnesium Sulfate (MgSO4) Solution - 6.0 ml
by New England Biolabs
Catalog #: B1003S

3.2 µl [M]4 Milimolar (mM)

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N gene primer mix
Catalog #: N/A

E gene primer mix
Catalog #: N/A

Saliva sample
Catalog #: N/A

Nuclease-free Water - 25 ml
by New England Biolabs
Catalog #: B1500S

Non-CRISPR nuclease
Catalog #: N/A

Manganese(II) chloride tetrahydrate
by Sigma Aldrich
Catalog #: M3634

0.8 µl **[M]0.5 Milimolar (mM)**

Non-primer oligos
Catalog #: N/A

Reporter probe 1
Catalog #: N/A

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Reporter Probe 2
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.

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