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SPOT1 assay

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1 Works for me

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SPOT

stlane2

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PROTOCOL CITATION

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41789

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

NAME CATALOG # VENDOR

Citation: Guanhua Xun, Huimin Zhao, stlane2 (09/08/2020). SPOT1 assay. https://dx.doi.org/10.17504/protocols.io.bk25kyg6

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.





& 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.



8 63 °C © 00:30:00 8 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
MgS04	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 μΜ	625 nM	3
Reporter probe 1	100 μΜ	156.25 nM	0.125
Reporter probe 2	100 μΜ	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

88

WarmStart RTx Reverse Transcriptase - 250 rxns

by New England Biolabs

Catalog #: M0380L

□1 μl

88

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

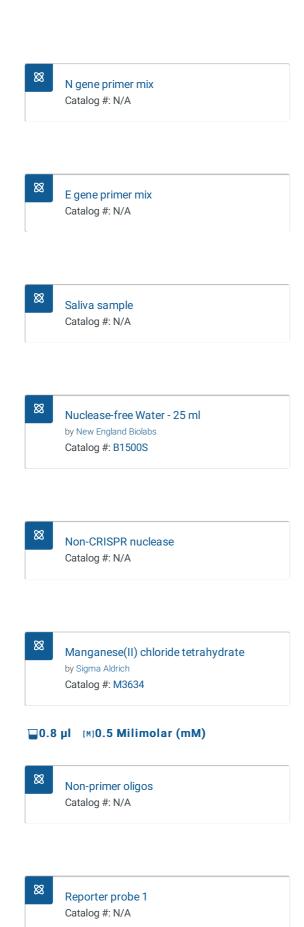
by New England Biolabs
Catalog #: B1003S

■3.2 µl [M]4 Milimolar (mM)

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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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