VERSION 1

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SPOT1 revised protocol V.1

Forked from [SPOT1 assay](#)

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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

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Protocol status: Working
 We use this protocol and it's working

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 45260

- 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.

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. 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:

	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
MgSO ₄	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 μ M	1.375 μ M	2
MnCl ₂	50 mM	0.5 mM	0.8
Non-primer oligos (total 6 oligos)	100 μ M	625 nM	3
Reporter probe 1	100 μ M	156.25 nM	0.125
Reporter probe 2	100 μ M	312.5 μ M	0.25
Nuclease-free water			44.025
Total			80

Bst 2.0 WarmStart DNA Polymerase - 8,000 units New England Biolabs Catalog #M05381
2 μ L
WarmStart RTx Reverse Transcriptase - 250 rxns New England Biolabs Catalog #M03801
1 μ L
Isothermal Amplification Buffer - 6.0 ml New England Biolabs Catalog #B0537S
8 μ L
Deoxynucleotide Solution Mix - 8 μ mol of each New England Biolabs Catalog #N0447S
5.6 μ L [M] 0.7 millimolar (mM)
Magnesium Sulfate (MgSO ₄) Solution - 6.0 ml New England Biolabs Catalog #B1003S
3.2 μ L [M] 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 μ L [M] 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 Insert capillary B into the SPOT1 device and press the "Start" button to initiate the 35-minute detection reaction.

35m

Equipment	
SPOT1 device	NAME
Incubating fluorometer	TYPE
University of Illinois	BRAND
N/A	SKU

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