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SPOT1 assay

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XPRIZE Rapid Covid Tes...

SPOT



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Protocol status: Working

We use this protocol in our workspace and it is working.



Created: September 08, 2020

Last Modified: September 08, 2020

Protocol Integer ID: 41789

Keywords: assay

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 **Catalog #N/A**
- ☒ Non-primer oligos **Catalog #N/A**
- ☒ E gene primer mix **Catalog #N/A**
- ☒ Isothermal Amplification Buffer - 6.0 ml **New England Biolabs Catalog #B0537S**
- ☒ N gene primer mix **Catalog #N/A**
- ☒ Reporter Probe 2 **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 **Catalog #N/A**
- ☒ Saliva sample **Catalog #N/A**
- ☒ QuickExtract DNA Extraction Solution **Lucigen Catalog #QE09050**



Protocol materials

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- ☒ E gene primer mix **Catalog #N/A**
- ☒ N gene primer mix **Catalog #N/A**
- ☒ Reporter Probe 2 **Catalog #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 **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 concentrati on	Final concentrati on	Amount (μ L)
	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 #M0538L**



2 μ L



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



1 μ L



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



8 μ L



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



5.6 μ L

[M] 0.7 millimolar (mM)



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



3.2 μ L

[M] 4 millimolar (mM)



N gene primer mix **Catalog #N/A**



E gene primer mix **Catalog #N/A**



Saliva sample **Catalog #N/A**



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



Non-CRISPR nuclease **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 **Catalog #N/A**



Reporter probe 1 **Catalog #N/A**



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.