Dec 05, 2020

SPOT2 revised protocol

Forked from SPOT2 protocol

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

SPOT

stlane2

DOI

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

PROTOCOL CITATION

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FORK NOTE

Applicable to both SPOT dashboard with water baths or with use of the SPOT carousel.

FORK FROM

Forked from SPOT2 protocol, stlane2

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MATERIALS TEXT

STEP MATERIALS

Magnesium Sulfate (MgSO4) Solution - 6.0 ml **New England Biolabs Catalog #B1003S** Step 4.2

E gene primer mix **Contributed by users Catalog #N/A** Step 4.2

Non-primer oligos **Contributed by users Catalog #N/A** Step 4.2

Bst 2.0 WarmStart DNA Polymerase - 8,000 units **New England Biolabs Catalog #M0538L** Step 4.2

WarmStart RTx Reverse Transcriptase - 250 rxns **New England Biolabs Catalog #M0380L** Step 4.2

Isothermal Amplification Buffer - 6.0 ml **New England Biolabs Catalog #B0537S** Step 4.2

Nuclease-free Water - 25 ml **New England Biolabs Catalog #B1500S** Step 4.2

Deoxynucleotide Solution Mix - 8 umol of each **New England Biolabs Catalog #N0447S** Step 4.2

N gene primer mix **Contributed by users Catalog #N/A** Step 4.2

Saliva sample **Contributed by users Catalog #N/A** Step 4.2

Manganese(II) chloride tetrahydrate **Sigma Aldrich Catalog #M3634** Step 4.2

Reporter probe 1 **Contributed by users Catalog #N/A** Step 4.2

Reporter Probe 2 **Contributed by users Catalog #N/A** Step 4.2

Non-CRISPR nuclease **Contributed by users Catalog #N/A** Step 4.2

Preparation of equipment and materials 1m

1 1. Gather the provided P-tubes (pretreatment tubes) and D-tubes (detection tubes). Refrigerate D-tubes until use. 1m
2. Gather microcaps to be used for saliva collection and sample transfer.
3. Plug in keyboard and power cable then power on the SPOT dashboard system.
4. **If using water baths:** Power on both sous vide water baths, setting one to 63°C and the other to 95°C.
5. **If using SPOT carousel device:** Plug the SPOT dashboard into the SPOT carousel with the included USB cable. Power on the SPOT carousel system.

SPOT testing protocol 40m

2 Prepare a new test kit and collect a patient saliva sample.

Citation: Huimin Zhao, stlane2 , Guanhua Xun (12/05/2020). SPOT2 revised protocol. https://dx.doi.org/10.17504/protocols.io.bqeymtfw

2.1 On the SPOT software main menu, press the "Prepare Kits" button to begin the testing process. Insert a P-tube into the SPOT dashboard, enter the patient's name, then press "Create" to link the capillary's RFID chip to the patient.

SPOT dashboard
Interactive data logging fluorometer

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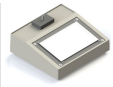

2.2 Provide the prepared P-tube to the patient along with a microcap for saliva collection. The saliva sample will be collected with the microcap then dispensed into the P-tube, containing QuickExtract DNA Extraction Solution (Lucigen).

QuickExtract DNA Extraction Solution **Lucigen Catalog #QE09050**

3 Perform sample pretreatment for 5 minutes at 95°C. 5m

- **If using water baths:** Place P-tube in floating rack and insert rack into sous vide water bath preheated to 95°C. Incubate for 5 minutes for sample pretreatment.
- **If using SPOT carousel device:** Place P-tube into carousel and close lid. On SPOT software, press the "Run Tests" button on the main menu then the "Pretreatment" button to perform a 95°C incubation for 5 minutes.

95 °C **00:05:00**

Thermal Immersion Cooker
Sous vide low cost waterbath

Wancle	**P00252**

SPOT carousel
Data logging incubating fluorometer

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4 Prepare detection reactions:

1. Pair P-tube with a D-tube (Detailed in substep 4.1).

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2. Transfer a small volume of pretreated sample to the D-tube (Detailed in substep 4.2).

4.1 Obtain an unused D-tube and pair it with a completed P-tube using the SPOT dashboard.

- On the SPOT dashboard main menu, click the "Pair Tubes" button.
- Tap the P-tube to the RFID antenna on the SPOT dashboard to populate patient information to the screen.
- Tap the unused D-tube to the RFID antenna on the SPOT dashboard. After the second RFID tag number populates, press the "Pair" button on the screen. Patient information assigned during preparation of a test kit will now be coupled to this D-tube.

4.2 Use the provided 20 µL microcap to transfer a small volume of pretreated saliva from the P-tube to the paired D-tube. Be sure to dispense the pretreated sample into ONLY the top layer of the D-tube, as shown in the diagram below. **Do not disturb the wax dividing layer during transfer of pretreated sample.**

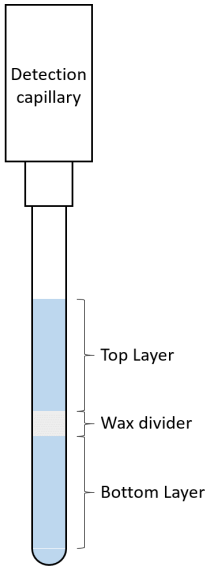

Diagram of D-tube layout. Pretreated saliva samples should be transferred into the top layer **only.** Disturbing the wax divider will result in a failed detection reaction.

D-tube contains the **SPOT assay mastermix:**

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A	B	C	D
	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**

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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 **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**

5 Run detection reaction: 35m

- **If using water baths:** Place D-tube in floating rack and insert rack into sous vide water bath preheated to 63°C and incubate for 30 minutes. After completion of the 63°C incubation, move the floating rack with D-tubes to sous vide water bath preheated to 95°C and incubate D-tubes for 5 minutes
- **If using SPOT carousel device:** Place D-tubes into carousel and close lid. On SPOT software, click the "Run Tests" button on the main menu then the "Detection" button to perform a full detection reaction (63°C for 30 minutes followed by a 95C for 5 minutes).

63 °C **00:30:00**

95 °C **00:05:00**

6 Measure test results:

- **If using water baths:** Press "Run Tests" on the main menu then of the SPOT dashboard. Transfer D-tubes one-by-one to the detection slot on SPOT dashboard then press the "Run" button to quantify test results for each D-tube. The integrated RFID chip on each D-tube will automatically link test result to the patient information entered previously and will be saved to the internal database.
- **If using SPOT carousel device:** After completion of the detection reaction, the SPOT carousel will automatically measure the fluorescent output of each D-tube present in the carousel. The diagnostic result will be linked to the appropriate patient using the integrated RFID antenna and saved to the internal database.

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SPOT dashboard

Interactive data logging fluorometer

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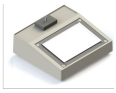

SPOT carousel

Data logging incubating fluorometer

University of Illinois at Urbana-Champaign	**N/A**

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