SPOT1 revised protocol V.1

Forked from SPOT1 assay

Guanhua Xun¹, Huimin Zhao¹, stlane2
¹University of Illinois at Urbana-Champaign

WORKS FOR ME
dx.doi.org/10.17504/protocols.io.bqfkmtkw

PROTOCOL CITATION

Guanhua Xun, Huimin Zhao, stlane2 2020. SPOT1 revised protocol. protocols.io
https://dx.doi.org/10.17504/protocols.io.bqfkmtkw

FORK NOTE

For use with the SPOT at-home device.

FORK FROM

Forked from SPOT1 assay, stlane2

LICENSE

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED
Dec 04, 2020

LAST MODIFIED
Dec 05, 2020

PROTOCOL INTEGER ID
45260

Citation: Guanhua Xun, Huimin Zhao, stlane2 (12/05/2020). SPOT1 revised protocol. https://dx.doi.org/10.17504/protocols.io.bqfkmtkw

This is an open access protocol distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
**STEP MATERIALS**

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

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.
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.](image)

**SPOT assay master mix:**
<table>
<thead>
<tr>
<th></th>
<th>Initial concentration</th>
<th>Final concentration</th>
<th>Amount (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper compartment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WarmStart® Bst 2.0</td>
<td>8000 units/mL</td>
<td>160 units/mL</td>
<td>2</td>
</tr>
<tr>
<td>WarmStart® RTx</td>
<td>15,000 units/mL</td>
<td>150 units/mL</td>
<td>1</td>
</tr>
<tr>
<td>Isothermal amplification buffer</td>
<td>10X</td>
<td>0.5X</td>
<td>8</td>
</tr>
<tr>
<td>dNTPs</td>
<td>10 mM</td>
<td>0.7 mM</td>
<td>5.6</td>
</tr>
<tr>
<td>MgSO4</td>
<td>100 mM</td>
<td>4 mM</td>
<td>3.2</td>
</tr>
<tr>
<td>N gene primer mix</td>
<td>10X</td>
<td>0.25X</td>
<td>2</td>
</tr>
<tr>
<td>E gene primer mix</td>
<td>10X</td>
<td>0.25X</td>
<td>2</td>
</tr>
<tr>
<td>Saliva samples</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Non-CRISPR nuclease</td>
<td>5 mg/mL or 55 μM</td>
<td>1.375 mM</td>
<td>2</td>
</tr>
<tr>
<td>MεCl2</td>
<td>50 mM</td>
<td>0.5 mM</td>
<td>0.8</td>
</tr>
<tr>
<td>Non-primer oligos</td>
<td>(total 6 oligos)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reporter probe 1</td>
<td>100 μM</td>
<td>156.25 nM</td>
<td>0.125</td>
</tr>
<tr>
<td>Reporter probe 2</td>
<td>100 μM</td>
<td>312.5 nM</td>
<td>0.25</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td></td>
<td></td>
<td>44.025</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>80</td>
</tr>
</tbody>
</table>

**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 [0.7 Millimolar (mM)]

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

3.2 μl [4 Millimolar (mM)]

**N gene primer mix** [Contributed by users](#)

**E gene primer mix** [Contributed by users](#)

**Saliva sample** [Contributed by users](#)

**Nuclease-free Water - 25 ml** [New England Biolabs Catalog #B1500S](#)
Insert capillary B into the SPOT1 device and press the "Start" button to initiate the 35-minute detection reaction.

<table>
<thead>
<tr>
<th>SPOT1 device</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubating fluorometer</td>
</tr>
<tr>
<td>University of Illinois</td>
</tr>
<tr>
<td>N/A</td>
</tr>
</tbody>
</table>

- $63 \degree C \ 00:30:00$

- $98 \degree C \ 00:05:00$

Result ("Positive"/"Negative"/"Inconclusive") will be displayed on SPOT1 device LCD screen after completion of detection reaction and the 1-minute cooling period.