XPRIZE SHINE - In-tube Fluorescent SARS-CoV-2 NP Test

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XPRIZE Rapid Covid Testing

Catherine Freije

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ABSTRACT

This protocol describes how to perform a SHINE in-tube fluorescent assay to detect SARS-CoV-2 RNA from a self-collected nasopharyngeal sample. This protocol is intended for point-of-care use. All enzymatic components are provided as a single-test freeze-dried pellet for shelf-stable storage, and all steps of the protocol are performed at ambient temperature. The protocol requires a transilluminator or another equivalent blue light emitting device. The protocol presented here is an improved version of the method presented in Arizti-Sanz J*, Freije CA*, et al. Integrated sample inactivation, amplification, and Cas13-based detection of SARS-CoV-2. bioRxiv (2020).

IMAGE ATTRIBUTION

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MATERIALS

MATERIALS

- FastAmp® Viral and Cell Solution for Covid-19 Testing Solution B Contributed by users Catalog #4633
- Custom nasopharyngeal swab and collection tube Contributed by users
- Reagent Mix A (In-tube SARS-CoV-2 resuspension mix) Contributed by users
- Lyophilized Reagent Mix B (In-tube SARS-CoV-2 detection mix) Contributed by users

STEP MATERIALS

- Custom nasopharyngeal swab and collection tube Contributed by users
- FastAmp® Viral and Cell Solution for Covid-19 Testing Solution B Contributed by users Catalog #4633
- Reagent Mix A (In-tube SARS-CoV-2 resuspension mix) Contributed by users
- Lyophilized Reagent Mix B (In-tube SARS-CoV-2 detection mix) Contributed by users

The necessary volume of FastAmp Viral and Cell Solution is provided in the tube used for sample collection. All enzymatic components, reagents, and compatible buffers required for SARS-CoV-2 detection are included within Lyophilized Reagent Mix B and are reconstituted with Reagent Mix A (see protocol for details). A transilluminator or equivalent blue light emitting device is needed to visualize the assay results. A smartphone or smart device is necessary for automated interpretation of the SARS-CoV-2 detection results using the HandLens application.
PROTOCOL MATERIALS

- Custom nasopharyngeal swab and collection tube *Contributed by users*
- FastAmp® Viral and Cell Solution for Covid-19 Testing Solution B *Contributed by users* Catalog #4633
- Reagent Mix A (In-tube SARS-CoV-2 resuspension mix) *Contributed by users*
- Lyophilized Reagent Mix B (In-tube SARS-CoV-2 detection mix) *Contributed by users*

SAFETY WARNINGS

> Please take care with potentially infectious sample material that does not come into contact with the provided viral lysis solution contained within the NP sample collection tube.

BEFORE START INSTRUCTIONS

Download the HandLens application on the user-provided smart device (smartphone, tablet, etc.). Clean workspace with disinfectant prior to starting the protocol.

Sample Collection and Viral Lysis

1. Open the nasopharyngeal (NP) collection tube and rotate the nasal swab (attached to the NP collection tube cap) 4 times around the inside of each nostril. Return the swab to the collection tube and cap the tube.

   *Nasopharyngeal collection tube contains necessary volume of FastAmp® Viral and Cell Solution.*

   - Custom nasopharyngeal swab and collection tube *Contributed by users*
   - FastAmp® Viral and Cell Solution for Covid-19 Testing Solution B *Contributed by users* Catalog #4633

2. Mix NP sample and FastAmp® Viral and Cell Solution by vortexing the closed sample collection
tube for 00:00:05.

3 Wait 00:05:00, incubating sample at Room temperature, before proceeding to Step 4.

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### SARS-CoV-2 Detection

4 Pipette 15 µL of Reagent Mix A into a single uncapped well of the 96-well plate containing lyophilized Reagent Mix B. Mix by pipetting up and down gently.

- Reagent Mix A (In-tube SARS-CoV-2 resuspension mix) Contributed by users
- Lyophilized Reagent Mix B (In-tube SARS-CoV-2 detection mix) Contributed by users

5 Add 5 µL sample-viral lysis mix to Reagent Mix A and B well. Mix by pipetting up and down gently. Recap sample.

6 Wait 01:30:00, incubating sample at Room temperature, before proceeding to Step 7.

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### In-tube Fluorescent Readout and Automated Analysis

7 Visualize the fluorescence of the sample using a transilluminator or equivalent blue light emitting device.

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**Equipment**

<table>
<thead>
<tr>
<th>13 x 12 cm mini Transilluminator</th>
<th>NAME</th>
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<tbody>
<tr>
<td>Clare Chemical Research</td>
<td>BRAND</td>
</tr>
<tr>
<td>DR22A</td>
<td>SKU</td>
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</tbody>
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With the user-provided smart device such as a smartphone, open the HandLens application and select in-tube as the test type.

Take a photo of the plate, and select upload. The result of the test will appear on the smart device screen.