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1 Works for me

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**ABSTRACT** 

This protocol describes how to perform a SHINE paper-based assay to detect SARS-CoV-2 RNA from a self-collected saliva sample. This protocol is intended for in-home 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 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).

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KEYWORDS

CRISPR, SARS-CoV-2, nucleic acid diagnostic

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#### MATERIALS

NAME	CATALOG #	VENDOR
Nalgene™ Dropper Bottles with Control Dispensing Tip, 4mL, white	2750-9125	Thermo Fisher
Screw cap tube 5 mL sterile	1188R46	Thomas Scientific
FastAmp® Viral and Cell Solution for Covid-19 Testing Solution B	4633	
Reagent Mix A (Paper-based SARS-CoV-2 resuspension mix)		
Lyophilized Reagent Mix B (Paper-based SARS-CoV-2 detection mix)		
HybriDetect – Universal Lateral Flow Assay Kit	MGHD 1	
Inoculating Loops and Needles Sterile 10 uL	12000-810	VWR International

#### STEPS MATERIALS

NAME	CATALOG #	VENDOR
FastAmp® Viral and Cell Solution for Covid-19 Testing Solution B	4633	
Screw cap tube 5 mL sterile	1188R46	Thomas Scientific
Lyophilized Reagent Mix B (Paper-based SARS-CoV-2 detection mix)		
Nalgene™ Dropper Bottles, 15mL, white	2751-9050	Thermo Fisher
Reagent Mix A (Paper-based SARS-CoV-2 resuspension mix)		
Inoculating Loops and Needles Sterile 10 uL	12000-810	VWR International
HybriDetect – Universal Lateral Flow Assay Kit	MGHD 1	

#### MATERIALS TEXT

The necessary volume of FastAmp Viral and Cell Solution is provided in the screw cap 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 smartphone or smart device is necessary for automated interpretation of the SARS-CoV-2 detection results using the HandLens application. The required volume of Reagent Mix A is provided in the Nalgene Dropper Bottle.

# 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 saliva sample collection tube.

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### BEFORE STARTING

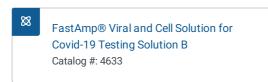
Download the HandLens application on the user-provided smart device (smartphone, tablet, etc.). Wash hands prior to starting the protocol.

Sample Collection and Viral Lysis

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1 Expel approximately one drop of saliva into the sample collection tube and cap the tube. Saliva collection tube contains necessary volume of FastAmp® Viral and Cell Solution.



Screw cap tube 5 mL sterile
by Thomas Scientific
Catalog #: 1188R46

- 2 Mix saliva sample and FastAmp® Viral and Cell Solution by shaking the closed sample collection tube for **© 00:00:10**
- 3 Wait © 00:05:00 incubating sample at & Room temperature before proceeding to Step 4.

### SARS-CoV-2 Detection

4 Add the entire volume in the dropper bottle containing Reagent Mix A to the tube containing lyophilized Reagent Mix B. Cap the tube.



- Reagent Mix A (Paper-based SARS-CoV-2 resuspension mix)
- Lyophilized Reagent Mix B (Paper-based SARS-CoV-2 detection mix)
- 5 Mix Reagent Mix A and B by shaking for approximately **© 00:00:10**.

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6 Dip the inoculation loop into the sample collection tube (a small layer of liquid should be contained within the loop).



- 7 Transfer liquid in inoculation loop to the Reagent Mix A and B tube by dipping the loop into tube and stirring for **© 00:00:05**. Remove and discard the inoculation loop and cap the tube.
- 8 Mix the sample combined with Reagent Mix A and B by shaking for approximately  $\odot$  00:00:10.
- 9 Wait © 01:30:00, incubating sample at & Room temperature, before proceeding to Step 10.

# Paper-based Readout and Automated Analysis

10 Open the Sample-Reagent Mix A and B tube and place the test strip into the liquid with the arrows on the test strip pointing upward and towards you.



- 11 Wait © 00:05:00 with sample at & Room temperature for visible horizontal bands to appear on the test strip.
- 12 With the user-provided smart device such as a smartphone, open the HandLens application and select paper-based as the test type.
- 13 Take a photo of the test strip, and select upload. The result of the test will appear on the smart device screen.