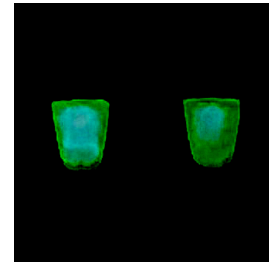


Sep 07, 2020

BW SARS-CoV-2 Laboratory Test

DOI

<https://dx.doi.org/10.17504/protocols.io.bkztkx6n>



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Protocol status: Working

We use this protocol and it's working

Created: September 07, 2020

Last Modified: September 07, 2020

Protocol Integer ID: 41747

Keywords: detecting sar, bw sar, equivalent of the lamp assay, laboratory, lamp assay, sar, test this protocol, assay, cov, similar protocol

Disclaimer

This protocol is not approved for clinical use.

Abstract

This protocol is the laboratory-based equivalent of the LAMP assay used for detecting SARS-CoV-2. A similar protocol is performed automatically within the Biology Works LLC at-home device.

Image Attribution

Photograph by Ivan Rueda.

Guidelines

This protocol is not approved at this time by any regulatory body for clinical use. It uses proprietary reagents available from Biology Works LLC and other vendors. Repetitive freezing of primers is not recommended; please store them at 4°C when not in use.



Materials

STEP MATERIALS

⊗ BW SARS-CoV-2 Primer Mix **Biology Works LLC Catalog #SARS-CoV-2 Primer Mix**

⊗ LAMP Fluorescent Dye **New England Biolabs Catalog #B1700AA**

⊗ WarmStart LAMP Kit (DNA and RNA) - 100 rxns **New England Biolabs Catalog #E1700S**

⊗ Guanidine HCL **Merck MilliporeSigma (Sigma-Aldrich) Catalog #G7294**

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⊗ BW SARS-CoV-2 Primer Mix **Biology Works LLC Catalog #SARS-CoV-2 Primer Mix**

⊗ LAMP Fluorescent Dye **New England Biolabs Catalog #B1700AA**

⊗ HyClone Water **GE Healthcare Catalog #SH30538LS**

⊗ WarmStart LAMP Kit (DNA and RNA) - 100 rxns **New England Biolabs Catalog #E1700S**

Protocol materials

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⊗ HyClone Water **GE Healthcare Catalog #SH30538LS**

Safety warnings

⚠ This protocol is not approved at this time by any regulatory body for clinical use. You must follow all safety protocols specified by each vendor, your laboratory, common sense, and regulatory bodies in your geography. All specimens, reagents, and equipment must be handled aseptically. Personal protective equipment, including appropriate gloves, clothing and protective eye ware must be worn at all times.



Before start

Acquire a nasal or saliva sample for use with this assay. The Chai Bio 1-step DNA/RNA Extraction Buffer (Cat. 05210S) has been demonstrated as being compatible with nasal swabs using this protocol. Saliva samples must be heat denatured at 95°C for 10 minutes.

Citation

S. Bhadra, T. E. Riedel, S. Lakhotia, N. D. Tran, and A. D. Ellington (2020)
. High-surety isothermal amplification and detection of SARS-CoV-2, including with crude enzymes. Biorxiv.

[10.1101/2020.04.13.039941](https://doi.org/10.1101/2020.04.13.039941)

[LINK](#)

Assay

33m 40s

- 1 Thaw reagents to room temperature  Room temperature

 WarmStart LAMP Kit (DNA and RNA) - 100 rxns **New England Biolabs** **Catalog #E1700S**

 Guanidine HCL **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #G7294**

 BW SARS-CoV-2 Primer Mix **Biology Works LLC** **Catalog #SARS-CoV-2 Primer Mix**

 LAMP Fluorescent Dye **New England Biolabs** **Catalog #B1700AA**

- 2 Vortex reagents  3000 rpm 3x

15s

Equipment

Vortexer

NAME

VWR

BRAND

97043-562

SKU

- 3 Briefly spin down components in a microcentrifuge  00:00:05

5s



Equipment

Microcentrifuge

NAME

VWR

BRAND

75993-410

SKU

- 4 Prepare master mix in a 1.5mL tube with the following sub-steps

Equipment

Seal-Rite 1.5mL Microcentrifuge Tube

NAME

USA Scientific

BRAND

1615-5510

SKU

- 4.1 Add  12.5 μ L to tube



WarmStart LAMP Kit (DNA and RNA) - 100 rxns **New England Biolabs** Catalog #E1700S

- 4.2 Add  .3125 μ L to tube



Guanidine HCL **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #G7294

- 4.3 Add  4 μ L to tube



BW SARS-CoV-2 Primer Mix **Biology Works LLC** Catalog #SARS-CoV-2 Primer Mix

4.4 Add  0.5 µL to tube

 LAMP Fluorescent Dye **New England Biolabs Catalog #B1700AA**

4.5 Add  2.8 µL to tube

 HyClone Water **GE Healthcare Catalog #SH30538LS**

4.6 Close tube cap

5 Vortex  3000 rpm 3x

15s

6 Briefly spin down components in a microcentrifuge  00:00:05

5s

7 Aliquot out 20uL into a PCR reaction plate

Equipment

Microamp Optical 96-well Reaction Plate

NAME

Thermo-Fisher

BRAND

N8010560

SKU

8 Dispense  5 µL of your sample solution into the well

9 Seal plate with film

**Equipment****Microamp Optical Adhesive Film**

NAME

Thermo-Fisher

BRAND

4311971

SKU

- 10 Centrifuge plate in plate centrifuge  500 rcf

Equipment**Centrifuge 5810 R**

NAME

Plate Centrifuge

TYPE

Eppendorf

BRAND

022625101

SKU

- 11 Place plate with reagents and sample into thermo-cycler

Equipment**7500 Real Time PCR System**

NAME

ABI

BRAND

4351104

SKU



12 Run assay on thermo-cycler with the following protocol (specific to the ABI 7500):

33m

1x 25 °C 00:02:00

60x 64.9 °C 00:00:02 and 65 °C 00:00:30

Record measurements

13 Read results

Citations

S. Bhadra, T. E. Riedel, S. Lakhotia, N. D. Tran, and A. D. Ellington. High-surety isothermal amplification and detection of SARS-CoV-2, including with crude enzymes

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