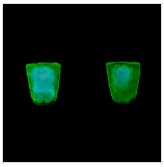

Sep 07, 2020

BW SARS-CoV-2 Laboratory Test

ivan [1], peter.marx [1]

[1]Biology Works LLC

1 Works for me dx.doi.org/10.17504/protocols.io.bkztkx6n

Biology Works LLC

peter.marx

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.

DOI

dx.doi.org/10.17504/protocols.io.bkztkx6n

PROTOCOL CITATION

ivan , peter.marx 2020. BW SARS-CoV-2 Laboratory Test. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bkztkx6n

IMAGE ATTRIBUTION

Photograph by Ivan Rueda.

CREATED

Sep 07, 2020

LAST MODIFIED

Sep 07, 2020

PROTOCOL INTEGER ID

41747

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.

STEPS MATERIALS

NAME	CATALOG #	VENDOR
BW SARS-CoV-2 Primer Mix	SARS-CoV-2 Primer Mix	Biology Works LLC
LAMP Fluorescent Dye	B1700AA	New England Biolabs
WarmStart LAMP Kit (DNA and RNA) - 100 rxns	E1700S	New England Biolabs
Guanidine HCL	G7294	Millipore Sigma
HyClone Water	SH30538LS	Ge Life Sciences

EQUIPMENT

NAME	CATALOG #	VENDOR
Vortexer	97043-562	VWR Scientific
Microcentrifuge	75993-410	
Microamp Optical 96-well Reaction Plate	N8010560	Thermo Fisher Scientific
Microamp Optical Adhesive Film	4311971	Thermo Fisher Scientific
Centrifuge 5810 R	022625101	
7500 Real Time PCR System	4351104	Applied Biosystems
Seal-Rite 1.5mL Microcentrifugre Tube	1615-5510	USA Scientific

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 asceptically. Personal protective equipment, including appropriate gloves, clothing and protective eye ware must be worn at all times.

DISCLAIMER:

This protocol is not approved for clinical use.

BEFORE STARTING

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.

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.
http://10.1101/2020.04.13.039941

Assay 33m 40s

1 Thaw reagents to room temperature **Room temperature**

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

Guanidine HCL
by Millipore Sigma
Catalog #: G7294

Citation: ivan , peter.marx (09/07/2020). BW SARS-CoV-2 Laboratory Test. https://dx.doi.org/10.17504/protocols.io.bkztkx6n

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

LAMP Fluorescent Dye
by New England Biolabs
Catalog #: B1700AA

2 Vortex reagents **3000 rpm** **3x** 15s

Vortexer
VWR 97043-562

3 Briefly spin down components in a microcentrifuge **00:00:05** 5s

Microcentrifuge
VWR 75993-410

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

Seal-Rite 1.5mL Microcentrifugre Tube
USA Scientific 1615-5510

4.1 Add **12.5 µl** to tube

Citation: ivan , peter.marx (09/07/2020). BW SARS-CoV-2 Laboratory Test. https://dx.doi.org/10.17504/protocols.io.bkztkx6n

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

4.2 Add **.3125 µl** to tube

Guanidine HCL
by Millipore Sigma
Catalog #: G7294

4.3 Add **4 µl** to tube

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

4.4 Add **0.5 µl** to tube

LAMP Fluorescent Dye
by New England Biolabs
Catalog #: B1700AA

4.5 Add **2.8 µl** to tube

HyClone Water
by Ge Life Sciences
Catalog #: SH30538LS

Close tube cap

Citation: ivan , peter.marx (09/07/2020). BW SARS-CoV-2 Laboratory Test. https://dx.doi.org/10.17504/protocols.io.bkztkx6n

4.6

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

Microamp Optical 96-well Reaction Plate
Thermo-Fisher **N8010560**

8 Dispense **5 µl** of your sample solution into the well

9 Seal plate with film

Microamp Optical Adhesive Film
Thermo-Fisher **4311971**

10 Centrifuge plate in plate centrifuge **500 rcf**

Centrifuge 5810 R
Plate Centrifure
Eppendorf **022625101**

11 Place plate with reagents and sample into thermo-cycler

7500 Real Time PCR System
ABI **4351104**

Citation: ivan , peter.marx (09/07/2020). BW SARS-CoV-2 Laboratory Test. https://dx.doi.org/10.17504/protocols.io.bkztkx6n

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

Citation: ivan , peter.marx (09/07/2020). BW SARS-CoV-2 Laboratory Test. https://dx.doi.org/10.17504/protocols.io.bkztkx6n