VERSION 2

SEP 10, 2020

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bk89kzz6

Protocol Citation: Albert Yu, Timothy A Brown, Jasmine Quynh Le, Kristina Galatsis, Michael Rosbash 2020. Rosbash/Janelia StickLAMP Protocol. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bk89kzz6> Version created by [Albert Yu](#)

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

Protocol status: Working
 We use this protocol and it's working

Created: Sep 10, 2020

Last Modified: Sep 10, 2020

PROTOCOL integer ID:
 41985

Rosbash/Janelia StickLAMP Protocol V.2

Albert Yu¹, Timothy A Brown², Jasmine Quynh Le¹, Kristina Galatsis¹, Michael Rosbash¹

¹Brandeis University; ²Janelia Research Campus

XPRIIZE Rapid Covid Testing

Albert Yu

ABSTRACT

A protocol for the detection of SARS-CoV-2 from saliva samples featuring a rapid purification step and a high-contrast colorimetric readout. Saliva is first inactivated using a 100x inactivation reagent consisting of 2.5M TCEP, 100 mM EDTA, 1.2N NaOH solution diluted to approximately 1x final concentration and heated to 95C for 5 minutes. RNA is rapidly purified and concentrated with magnetic beads in a PEG/NaCl-based buffer using a 3D-printed magnetic stick that enables selective separation of beads without carryover of saliva contaminants. Beads are eluted directly into an RT-LAMP reaction mix, which uses a novel high contrast dye that turns from purple to clear when acidified by nucleic acid amplification products that enables unambiguous identification of successful amplification. This protocol is sensitive down to 1 copy/μl of SARS-CoV-2 in 300 μl of saliva. This degree of sensitivity enables faithful detection of SARS-CoV-2 even in pooled samples.

MATERIALS

MATERIALS

⊗ NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #53014**

⊗ Twist synthetic SARS-CoV-2 RNA control **Twist Bioscience Catalog #Mt007544.1**

⊗ SARS-CoV-2 Master Mix **Contributed by users**

⊗ Actin Master Mix **Contributed by users**

⊗ 100x Inactivation Reagent **Contributed by users**

⊗ Bead Mix **Contributed by users**

⊗ Magnetic Tips **Contributed by users**

⊗ Heat Block at 65C **Contributed by users**

⊗ Heat Block at 95C **Contributed by users**

⊗ Magnetic Stick **Contributed by users**

STEP MATERIALS

-

⊗ Magnetic Tips **Contributed by users**

⊗ NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #53014**

⊗ SARS-CoV-2 Master Mix **Contributed by users**

⊗ Actin Master Mix **Contributed by users**

⊗ Water **Contributed by users**

⊗ Twist synthetic SARS-CoV-2 RNA control **Twist Bioscience Catalog #Mt007544.1**

100x Inactivation Reagent

2.5M TCEP

150mM EDTA

1.2N NaOH

SARS-CoV-2/Actin Master Mix

12.5µl SARS-CoV-2/Actin Buffer/Dye/Primer Mix (Currently only available from us)

0.5µl WarmStart RTx NEB M0380L

1µl Bst2.0 NEB M0537L

11µl H₂O

Primers used

See <https://docs.google.com/spreadsheets/d/11n-9754VqtsXszTC2tUxFq-gKlGgjPL-KtKqXVevH4/edit#gid=0>

Bead Mix

See https://ethanomics.files.wordpress.com/2012/08/serapure_v2-2.pdf with 300µl beads instead of 1000µl

SAFETY WARNINGS

Do not open up PCR tubes after amplification.

BEFORE START INSTRUCTIONS

Prepare:

Saliva collection kit (2.0ml Tube and funnel provided by us, or your own saliva collection device from standard labware, such as 1.5ml, 5ml, 15ml, or 50ml tubes. Saliva samples >1ml will likely have to be subsampled)

Magnetic stick

1 magnetic tip per sample

Bead mix: Let bead mix come to room temperature for 20 minutes prior to use, and ensure beads are suspended in solution by vortexing or pipetting up and down

130mM NaCl

SARS-CoV-2 Master mix

Actin Master mix

Saliva Collection

- 1 Instruct patient to avoid food, drink, toothbrushing, and nasal sprays for a minimum of 00:30:00 prior to sample collection
- 2 Begin pooling saliva in your mouth. Saliva production can be stimulated by thinking about food, or about the saliva collection itself.
- 3 Gently expel saliva into the funnel, tapping to collect in the tube, until amount of saliva is approximately flush with the base of the funnel 750 μ L Approximately

Inactivation

5m

- 4 Add inactivation reagent to approximately 1x final concentration. Reaction is tolerant of between 0.7x to 2x final concentration. 7.5 μ L Approximately
100x Inactivation Reagent **Contributed by users**

5 Invert 40 times to mix

6 Heat tube to approximately **🌡️ 95 °C** for **🕒 00:05:00** . Viral RNA release is similar between 93-98C. Use tube clip to prevent popping.

5m

Equipment

ThermoMixer

NAME

Benchtop Incubator

TYPE

Eppendorf

BRAND

5382000023

SKU

<https://online-shop.eppendorf.us/US-en/Temperature-Control-and-Mixing-44518/Instruments-44519/Eppendorf-ThermoMixerC-PF-19703.html>

LINK

Any heat block will suffice

SPECIFICATIONS

7 Remove tube from heat and let rest at **🌡️ Room temperature** for at least **🕒 00:03:00** **OR** **🌡️ On ice** for at least **🕒 00:00:30** .

Quick Purification and LAMP Reaction

1h

8 While tube is resting, aliquot **🧴 25 µL** SARS-CoV-2 mastermix and **🧴 25 µL** Actin mastermix

to separate wells of PCR strip tube, 96-well plate, or 1.5ml tube per sample On ice .

Per run, prepare two additional 25 μ L SARS-CoV-2 mastermixes for positive and negative controls.

SARS-CoV-2 Master Mix **Contributed by users**

Actin Master Mix **Contributed by users**

Step 8 includes a Step case.

If pooling

step case

If pooling

Prepare one 25ul SARS-CoV-2 reaction and one 25ul Actin reaction per 5 samples

- 9 Add approximately 0.7x volumes of bead mix - 525 μ L Approximately . Sample is tolerant of between 0.7x-1.2x volumes of bead mix. Pipette up and down to mix.

Bead Mix **Contributed by users**

Step 9 includes a Step case.

If pooling

If you would like to preserve some sample

step case

10m

If pooling

Remove 60ul of inactivated saliva from 5 samples and add to a single tube, for a total of 300 μ L.

Add 210 μ L bead mix to pooled tube.

- 10 Let stand at Room temperature for 00:03:00

- 11 Cap magnetic stick with a clean tip and dip in bead/sample mix for 00:02:00 , dipping up and down 5 times every 00:00:30 . Meanwhile, prepare 500 μ L 130mM NaCl in a separate 1.5ml or 2ml tube.

2m

Equipment

Magnetic Stick

Rosbash/Brown

None

Magnetic stick used for bead purifications

NAME

BRAND

SKU

SPECIFICATIONS

Magnetic Tips **Contributed by users**

NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #53014**

12 Remove magnetic stick from sample and swirl in clean 130mM NaCl solution for 00:00:05 . **5s**
Discard NaCl solution.

13 Remove magnetic stick from wash sample and place in SARS-CoV-2 mix for 00:00:30 **30s**

14 Remove magnetic stick from SARS-CoV-2 mix and place in Actin mix for 00:00:30 **30s**

15 Add 5 μ L water to additional SARS-CoV-2 Mix (negative control) and 5 μ L synthetic Twist SARS-CoV-2 positive RNA control to additional SARS-CoV-2 Mix, prepared in Step 8.

Water **Contributed by users**

Twist synthetic SARS-CoV-2 RNA control **Twist Bioscience Catalog #Mt007544.1**

16

Cap tubes and place on

🌡️ 65 °C

 heating apparatus for

🕒 00:40:00

 .

40m

If using a thermal cycler, run with the following program:

65C for 40 minutes
4C indefinitely

Equipment

ThermoMixer

Benchtop Incubator

Eppendorf

5382000023

<https://online-shop.eppendorf.us/US-en/Temperature-Control-and-Mixing-44518/Instruments-44519/Eppendorf-ThermoMixerC-PF-19703.html>

Any heat block will suffice

NAME

TYPE

BRAND

SKU

LINK

SPECIFICATIONS

17

Remove tubes from heating apparatus and examine color change.

Positive		Negative		Inconclusive			
SARS-CoV-2	Actin	SARS-CoV-2	Actin	SARS-CoV-2	Actin

SARS-CoV-2	Actin			Negative Control

				Positive Control

18 If a positive sample is found when pooling, re-test pooled samples individually.