

Sep 08, 2020

Version 1

Rosbash/Janelia StickLAMP Protocol V.1

DOI

dx.doi.org/10.17504/protocols.io.bk33kyqn



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

¹Brandeis University; ²Janelia Research Campus

XPRIZE Rapid Covid Tes...



Albert Yu

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.bk33kyqn>

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.bk33kyqn](https://dx.doi.org/10.17504/protocols.io.bk33kyqn)

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: September 08, 2020

Last Modified: September 08, 2020

Protocol Integer ID: 41819

Keywords: saliva sample, saliva contaminant, carryover of saliva contaminant, saliva, μ l of saliva, rapid purification step, nucleic acid amplification product, detection of sar, rna, faithful detection of sar, selective separation of bead, novel high contrast dye, colorimetric readout


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/ μ l of SARS-CoV-2 in 300 μ l 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

 Actin Master Mix

 100x Inactivation Reagent

 Bead Mix

 Magnetic Tips

 Heat Block at 65C

 Heat Block at 95C

 Magnetic Stick

STEP MATERIALS

 SARS-CoV-2 Master Mix


 Actin Master Mix

 Magnetic Tips

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

 Water

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

 Bead Mix

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

0.5µl WarmStart RTx NEB M0380L

1µl Bst2.0 NEB M0537L

11µl H₂O

Bead Mix

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




Protocol materials







- ✕ 100x Inactivation Reagent
- ✕ Heat Block at 65C
- ✕ Twist synthetic SARS-CoV-2 RNA control **Twist Bioscience Catalog #Mt007544.1**
- ✕ Water
- ✕ Actin Master Mix
- ✕ Magnetic Tips
- ✕ Bead Mix
- ✕ Bead Mix
- ✕ Heat Block at 95C
- ✕ Magnetic Stick
- ✕ NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #53014**
- ✕ SARS-CoV-2 Master Mix
- ✕ NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #53014**
- ✕ SARS-CoV-2 Master Mix
- ✕ Actin Master Mix
- ✕ Magnetic Tips
- ✕ Twist synthetic SARS-CoV-2 RNA control **Twist Bioscience Catalog #Mt007544.1**
- ✕ 100x Inactivation Reagent
- ✕ SARS-CoV-2 Master Mix
- ✕ Actin Master Mix
- ✕ Bead Mix
- ✕ Magnetic Tips
- ✕ NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #53014**
- ✕ Water
- ✕ Twist synthetic SARS-CoV-2 RNA control **Twist Bioscience Catalog #Mt007544.1**

Troubleshooting

Safety warnings

 Do not open up PCR tubes after amplification.



- 1 Instruct patient to avoid food, drink, toothbrushing, and nasal sprays for a minimum of 30 minutes prior to sample collection  00:30:00
- 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
- 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
- 5 Invert 40 times to mix
- 6 Heat tube to approximately 95C for 5 minutes. Viral RNA release is similar between 93-98C. Use tube clip to prevent popping.  95 °C  00:05:00

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 3 minutes or on ice for at least 30 seconds.

 00:03:00

 Room temperature

- 8 While tube is resting, aliquot 25 μ L SARS-CoV-2 mastermix and 25 μ L Actin mastermix to separate wells of PCR strip tube, 96-well plate, or 1.5ml tube per sample.

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

 SARS-CoV-2 Master Mix

 25 μ L

 Actin Master Mix



25 µL

STEP CASE

If pooling

From 1 to 10 steps

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

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

Bead Mix

525 µL Approximately

STEP CASE

If pooling

9 steps

Remove 60ul of inactivated saliva from 5 samples and add to a single tube, for a total of 300µl. Add 210µl bead mix to pooled tube.

- 10 Let stand at room temperature for 3 minutes 00:03:00

10m

- 11 Cap magnetic stick with a clean tip and dip in bead/sample mix for two minutes, swirling every 30 seconds. Meanwhile, prepare 500µl 130mM NaCl in a separate 1.5ml or 2ml tube. 00:02:00

2m

Equipment

Magnetic Stick

NAME

Rosbash/Brown

BRAND


None


SKU


Magnetic stick used for bead purifications

SPECIFICATIONS


 Magnetic Tips NaCl Merck MilliporeSigma (Sigma-Aldrich) Catalog #53014 500 µL

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

13 Remove magnetic stick from wash sample and swirl in SARS-CoV-2 mix for 30 seconds.  00:00:30 30s

14 Remove magnetic stick from SARS-CoV-2 mix and swirl in Actin mix for 30 seconds.  00:00:30 30s

15 Add 5 µl water to additional SARS-CoV-2 Mix (negative control) and 5µl positive control to additional SARS-CoV-2 Mix, prepared in Step 8.

 Water 5 µL Twist synthetic SARS-CoV-2 RNA control Twist Bioscience Catalog #Mt007544.1 5 µL

16 Cap tubes and place on 65C heating apparatus for 40 minutes. 40m

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

65C for 40 minutes

4C indefinitely

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



🌡️ 65 °C

🕒 00:40:00

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.