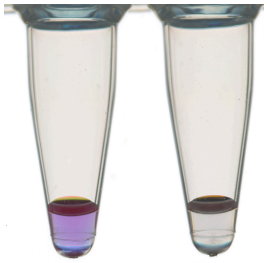
Sep 10, 2020 Version 2

Rosbash/Janelia StickLAMP Protocol V.2



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

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XPRIZE Rapid Covid Tes...

Albert Yu

OPEN ACCESS

DOI: https://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**

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

Created: September 10, 2020

Last Modified: September 10, 2020

Protocol Integer ID: 41985

Keywords: saliva sample, saliva contaminant, carryover of saliva contaminant, saliva, µl of saliva, rapid purification step, nucleic acid amplification product, 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/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

Actin Master Mix

100x Inactivation Reagent

Bead Mix

Magnetic Tips

Heat Block at 65C

Heat Block at 95C

Magnetic Stick

STEP MATERIALS

-

-

Magnetic Tips

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

SARS-CoV-2 Master Mix

Actin Master Mix

Water

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-_gKIGgjPL-KtKqXVevH4/edit#gid=0

Bead Mix

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

Protocol materials

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

Before start

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 µL 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 µL Approximately

100x Inactivation Reagent

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 .

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 µL SARS-CoV-2 mastermixes for positive and negative controls.

SARS-CoV-2 Master Mix

Actin Master Mix

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 - 525 µL Approximately . Sample is tolerant of between 0.7x-1.2x volumes of bead mix. Pipette up and down to mix.
Bead Mix

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 00:03:00 10m

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 µL 130mM NaCl in a separate 1.5ml or 2ml tube. 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

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

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

Water

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

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

17 Remove tubes from heating apparatus and examine color change.

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