Version 2

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Rosbash/Janelia StickLAMP Protocol V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bk89kzz6

XPRIZE Rapid Covid Testing

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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.

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MATERIALS

NAME	CATALOG #	VENDOR
NaCl	53014	Sigma Aldrich
Twist synthetic SARS-CoV-2 RNA control	Mt007544.1	Twist Bioscience
SARS-CoV-2 Master Mix		
Actin Master Mix		
100x Inactivation Reagent		
Bead Mix		

Citation: Albert Yu, Tim Brown, Jasmine Quynh Le, Kristina Galatsis, Michael Rosbash (09/10/2020). Rosbash/Janelia StickLAMP Protocol. https://dx.doi.org/10.17504/protocols.io.bk89kzz6

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NAME	CATALOG #	VENDOR
Magnetic Tips		
Heat Block at 65C		
Heat Block at 95C		
Magnetic Stick		

STEPS MATERIALS

NAME	CATALOG #	VENDOR
100x Inactivation Reagent		
Bead Mix		
Magnetic Tips		
NaCl	53014	Sigma Aldrich
SARS-CoV-2 Master Mix		
Actin Master Mix		
Water		
Twist synthetic SARS-CoV-2 RNA control	Mt007544.1	Twist Bioscience

MATERIALS TEXT

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

EQUIPMENT

NAME	CATALOG #	VENDOR
ThermoMixer	5382000023	
Magnetic Stick	None	

SAFETY WARNINGS

Do not open up PCR tubes after amplification.

BEFORE STARTING

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

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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

ThermoMixer
Benchtop Incubator
Eppendorf **5382000023**
Any heat block will suffice

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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 µl** SARS-CoV-2 mastermixes for positive and negative controls.

SARS-CoV-2 Master Mix

Actin Master Mix

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 µl Approximately** . Sample is tolerant of between 0.7x-1.2x volumes of bead mix. Pipette up and down to mix.

Bead Mix
View

Step 9 includes a Step case.
If pooling

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If you would like to preserve some sample

step case

If pooling

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

Magnetic Stick

Rosbash/Brown **None**

Magnetic stick used for bead purifications

Magnetic Tips

NaCl

by 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.

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Water

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

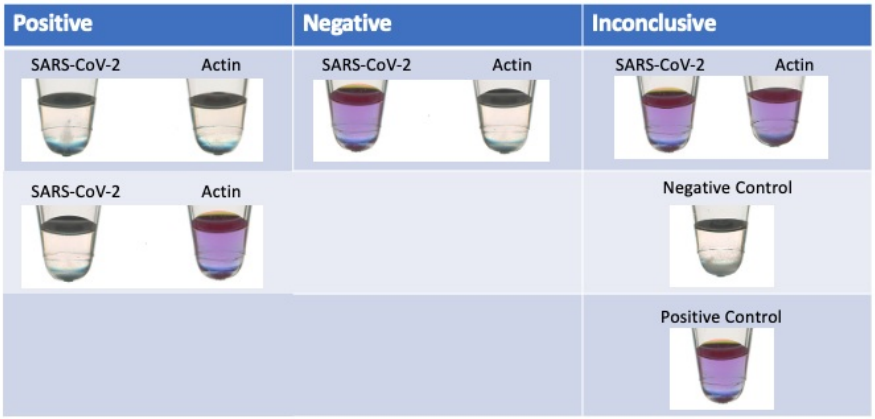
16 Cap tubes and place on **65 °C** heating apparatus for **00:40:00** . 40m

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

65C for 40 minutes
4C indefinitely

ThermoMixer
Benchtop Incubator
Eppendorf 5382000023
Any heat block will suffice

17 Remove tubes from heating apparatus and examine color change.



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

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