

Version 1

Sep 08, 2020

# Rosbash/Janelia StickLAMP Protocol V.1

Albert Yu<sup>1</sup>, Tim Brown<sup>2</sup>, Jasmine Quynhe Le<sup>1</sup>, Kristina Galatsis<sup>1</sup>, Michael Rosbash<sup>1</sup><sup>1</sup>Brandeis University; <sup>2</sup>Janelia Research Campus

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Works for me

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

XPRIZE 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.

## DOI

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

## PROTOCOL CITATION

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41819


## MATERIALS

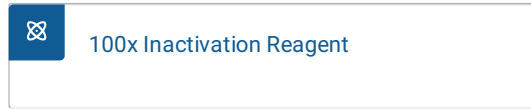
NAME	CATALOG #	VENDOR
NaCl	53014	Sigma Aldrich
Twist synthetic SARS-CoV-2 RNA control	Mt007544.1	Twist Bioscience
SARS-CoV-2 Master Mix		
Actin Master Mix		
100x Inactivation Reagent		
Bead Mix		
Magnetic Tips		
Heat Block at 65C		

NAME	CATALOG #	VENDOR
Heat Block at 95C		
Magnetic Stick		
STEPS MATERIALS		
NAME	CATALOG #	VENDOR
100x Inactivation Reagent		
SARS-CoV-2 Master Mix		
Actin Master Mix		
Magnetic Tips		
NaCl	53014	Sigma Aldrich
Water		
Twist synthetic SARS-CoV-2 RNA control	Mt007544.1	Twist Bioscience
Bead Mix		
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		
0.5µl WarmStart RTx NEB M0380L		
1µl Bst2.0 NEB M0537L		
11µl H2O		
Bead Mix		
See <a href="https://ethanomics.files.wordpress.com/2012/08/serapure_v2-2.pdf">https://ethanomics.files.wordpress.com/2012/08/serapure_v2-2.pdf</a> 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.		



- 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 µl Approximately**

- 4 Add inactivation reagent to approximately 1x final concentration. Reaction is tolerant of between 0.7x to 2x final concentration.  **7.5 µl Approximately**



- 5 Invert 40 times to mix

- 6 Heat tube to approximately 95°C for 5 minutes. Viral RNA release is similar between 93-98°C. Use tube clip to prevent<sup>5m</sup> popping.  **95 °C**  **00:05:00**




- 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 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.

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

 SARS-CoV-2 Master Mix

 25 µl

 Actin Master Mix

 25 µl

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

 Bead Mix  
View

 525 µl Approximately

Step 9 includes a Step case.



#### If pooling


#### 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 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, <sup>2m</sup>prepare 500µl 130mM NaCl in a separate 1.5ml or 2ml tube.  00:02:00




**Magnetic Stick**

**Rosbash/Brown**      **None**

Magnetic stick used for bead purifications



**Magnetic Tips**




**NaCl**


by 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. 5s  
 **00:00:05**

- 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**

by 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



ThermoMixer  
Benchtop Incubator

Eppendorf 5382000023 [Link](#)

Any heat block will suffice



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.