



Dec 04, 2020

# COVID19 RTLAMP Assay\_Nov\_2020

Forked from [COVID19 RTLAMP Assay](#)Arun Manoharan Arunprimediscoveriescom<sup>1</sup>, [Eugene Joseph](#)<sup>1</sup><sup>1</sup>Prime Discoveries

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*Works for me*

This protocol is published without a DOI.

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Eugene Joseph

## PROTOCOL CITATION

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**protocols.io**  
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## FORK NOTE

## FORK FROM

Forked from [COVID19 RTLAMP Assay, matt](#)

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## CREATED

Nov 27, 2020

## LAST MODIFIED

Dec 04, 2020

## PROTOCOL INTEGER ID

44932

## MATERIALS TEXT

### MATERIALS

☒ [RNAse and DNase-free 1.5ml tubes](#) Contributed by users☒ [Tubes, strips of 8 Thermo](#)**Fisher Catalog #AB0452**

### STEP MATERIALS

☒ [Binding Solution](#) Contributed by users In 3 steps☒ [Binding Solution](#) Contributed by users In 3 steps☒ [Wash Solution](#) Contributed by users In 2 steps☒ [Resuspension Buffer](#) Contributed by users In 2 steps☒ [Reaction Mix](#) Contributed by users In 2 steps☒ [Primer Mix](#) Contributed by users In 2 steps☒ [Positive Control](#) Contributed by users In 2 steps☒ [Lysis Buffer](#) Contributed by users In 3 steps☒ [Lysis Buffer](#) Contributed by users In 3 steps

## SAFETY WARNINGS

DISCLAIMER:

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

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BEFORE STARTING

Heat incubator

Sample Lysis / Inactivation 32m

- 1 Thaw the lysis Buffer on ice 1 to 3 hours before starting the experiment. It is recommended to aliquot the lysis buffer in small volumes and use it as needed to avoid excessive freeze-thawing cycles.

- 2 Thaw samples on ice if necessary and briefly vortex. Centrifuge the samples at 1000xg for 2 minutes to pellet solid<sup>32m</sup> debris. Alternatively, when completely thawed, samples can be left on ice for 30 minutes. Solid debris should be visible at the bottom of the tube.

🕒 00:02:00

or

🕒 00:30:00

- 3 **If using a Heat Block or water bath proceed to step 4. If using a thermal cycler proceed to step 9. If following the full method/sample purification proceed to step 12.**

#### 4 SAMPLES PREPARATION FOR INCUBATION IN A HEAT BLOCK OR WATER BATH

Transfer 100 µL of the sample Input Material (or clarified supernatant if working with fresh saliva) to a new microcentrifuge tube (1.5 mL capacity) containing an equal volume of lysis buffer (e.g. for 100 µL of sample add 100 µL of lysis buffer). Mix the obtained solution a few times by pipetting up and down. Label the tube with sample ID and other relevant information.

🔗 Lysis Buffer Contributed by users

📄 100 µl Lysis Buffer

📄 100 µl Clarified Sample

- 5 Lock the microcentrifuge lid using a lid lock (alternatively, an Eppendorf Safe-Lock tube can be used) to prevent tubes from popping open when exposed to high temperature.
- 6 Set the temperature of a heat block or water bath at 95°C. When the temperature has reached 95°C, place the tubes in the heat block or water bath and incubate for 5 minutes.

🕒 00:05:00

ThermoMixer  
Benchtop Incubator

Eppendorf 5382000023



Any heat block will suffice



- 7 Carefully transfer the tubes to ice or a 4°C cold block for 30 sec, or let sit at Room Temperature for 5 min.

5m 30s

🕒 00:00:30 4 °C

Or

🕒 00:05:00 Room temperature

- 8 Centrifuge the tubes for 3 sec at 2000xg to spin down any condensation using a benchtop mini centrifuge. Samples are ready for the RT-LAMP reaction. Proceed to step 26.

Mini-centrifuge  
Centrifuge

Fisher S67601B



Any standard mini centrifuge with adapters for different tube sizes will suffice



## 9 SAMPLES PREPARATION FOR INCUBATION IN A THERMAL CYCLER

Transfer 20ul of the sample input material (or clarified supernatant if Fresh Saliva) to a new 200 µL tube containing an equal volume of lysis buffer (e.g. for 20 µL of sample add 20 µL of lysis buffer). Mix the obtained solution a few times by pipetting up and down. Label the tube with sample ID and other relevant information.

🔗 Lysis Buffer Contributed by users

📄 20 µl Lysis Buffer

📄 20 µl Clarified Sample

- 10 Create a Method on the Thermal Cycler using the following steps:

5m

Step 1: 🔥 95 °C for 🕒 00:05:00

Step 2: Hold at 🔥 4 °C

Lid temperature: 🔧 **105 °C**

When ready run the program.

SimpliAmp Thermal Cycler  
PCR

Applied Biosystems A24811 [↗](#)

Any standard PCR thermocycler will suffice



- 11 Centrifuge the tubes for 3 sec at 2000xg to spin down any condensation using a benchtop mini centrifuge. Samples are ready for the RT-LAMP reaction. Proceed to step 26.

#### Sample Purification / Concentration

- 12 Preheat the heat block or water bath as instructed in step 6.

- 13 Vortex to mix the binding solution so there is no sediment.

[🔗 Binding Solution](#) Contributed by users

- 14 To each sample tube, add 800 ul of Binding Solution

#### **800 µl Binding Solution**

- 15 Let the Tubes sit at Room Temperature for 10 min, every 2 min invert the tube to suspend the Binding Solution. 10m

🕒 **00:10:00** at 🔧 **Room temperature**

- 16 Centrifuge for 3 sec at 2000xg to pellet the Binding Reagent in the Binding Solution.

Note: A white smear at the bottom of the tube should be present. The Binding Reagent should adhere to the bottom of the tube with a short spin on a simple benchtop centrifuge. Depending on tube type and input sample viscosity, a longer spin may be required until it is sufficiently pelleted.

Mini-centrifuge  
Centrifuge

Fisher S67601B



Any standard mini centrifuge with adapters for different tube sizes will suffice



- 17 Remove the supernatant with a 1ml Pipette taking care not to disturb the pellet.

**1000 µl Supernatant**

- 18 Add 1ml of Wash Solution, wash the pellet by closing and inverting the tube several times.

**Wash Solution Contributed by users**

**1000 µl Wash Solution**

- 19 Centrifuge for 3 sec at 2000xg to pellet the Binding Reagent in the Binding Solution

3s

Mini-centrifuge  
Centrifuge

Fisher S67601B



Any standard mini centrifuge with adapters for different tube sizes will suffice



**00:00:03**

- 20 Remove the supernatant with a 1ml Pipette taking care not to disturb the pellet.

**1000 µl Wash Supernatant**

- 21 Centrifuge for 3 sec at 2000xg to collect any residual Wash Solution.

3s

Mini-centrifuge  
Centrifuge

Fisher S67601B [↗](#)

Any standard mini centrifuge with adapters for different tube sizes will suffice



 00:00:03

- 22 Remove the residual supernatant with a 200ul Pipette taking care not to disturb the pellet.

 **200 µl Wash Supernatant**

- 23 Air dry in a clean ventilated area until the Binding Reagent is dry, it should appear opaque and not reflective, air drying<sup>15m</sup> should take 10-15 min, some sample types may take longer to dry, do not let drying take longer than 30 min.

Note: If the lab is equipped, air drying should take place in a ventilated air cabinet to prevent cross-contamination. To speed up the air drying, if available it is possible to use a Centrifuge with open tube lids, or a heated vacuum concentrator. If using these it would take less than 15 minutes as it should be evaporating less than 5-10ul of Wash Solution.

 00:15:00

- 24 To each tube add 10ul of Resuspension Buffer, pipette mix to resuspend the pellet. Depending on the Sample Input, the pellet may resuspend easily or stay as clumps that will take more pipetting to break apart.

 **Resuspension Buffer Contributed by users**

 **5 µl Resuspension Buffer**

- 25 Vortex until the Binding Reagent is resuspended, you can briefly centrifuge for 3 sec at 2000xg to collect droplets from the sides of the tube.

Note: As the sample contains extracted resuspended RNA, the RT-LAMP reaction should proceed the same day. The extracted samples should not be stored or frozen as it would impact the RNA integrity.

Mini-centrifuge  
Centrifuge

Fisher S67601B [↗](#)

Any standard mini centrifuge with adapters for different tube sizes will suffice



## RT-LAMP REACTION

30s

- 26 Prepare the Real-time PCR instrument, and work on ice for the following steps. Calculate the number of samples being run and make sure to include a positive control and the assay negative control (water only).

QuantStudio 3 Real-Time PCR System  
Real-Time PCR

Applied Biosystem A28567  
4 excitation filters (450–600 nm)  
4 emission filters (500–640 nm)



- 27 Prepare the Assay mix by adding Reaction Mix and Primer mix according to the number of samples being run.

☒ Reaction Mix Contributed by users

☒ Primer Mix Contributed by users

 12 µl Reaction Mix per sample

 3 µl Primer Mix per Sample

- 28 Transfer 15ul of Assay mix into the Assay wells.

 15 µl Assay Mix

- 29 Transfer 5ul of each sample into the appropriate Assay well, pipette mix.

 5 µl Sample

- 30 Add the Positive Control to the Plate or Strip Tube.

☒ Positive Control Contributed by users

 5 µl Positive Control

- 31 Transfer 5ul of UltraPure Distilled water into the appropriate Negative Control well.

### **5 µl UltraPure Distilled Water**

32 Seal the plate and run it in the Real-Time PCR instrument with the following settings :

30s

#### **Channels and Dye**

FAM - No Quencer = Assay Target

Cy3 - No Quencher = Control Target

PCR STAGE

 **00:00:30**  **68.5 °C** for 80 Cycles

Reaction Volume 20 ul

### **20 µl Reaction Volume**

Be sure that the capture/collect image option is selected at the end of the temperature stage (usually a small icon showing a camera should be selected below the temperature settings).