Dec 05, 2020

COVID19 RTLAMP Assay_Nov_2020

Forked from COVID19 RTLAMP Assay_Nov_2020

Arun Manoharan Arunprimediscoveriescom[1], Eugene Joseph[1]

[1]Prime Discoveries

1 *Works for me* This protocol is published without a DOI.

E Eugene Joseph

PROTOCOL CITATION

Arun Manoharan Arunprimediscoveriescom, Eugene Joseph 2020. COVID19 RTLAMP Assay_Nov_2020. **protocols.io** https://protocols.io/view/covid19-rtlamp-assay-nov-2020-bqf2mtqe

FORK NOTE

FORK FROM

Forked from COVID19 RTLAMP Assay_Nov_2020, Eugene Joseph

LICENSE

CREATED

Dec 05, 2020

LAST MODIFIED

Dec 05, 2020

PROTOCOL INTEGER ID

45274

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** Step 13

Binding Solution **Contributed by users** Step 13

Wash Solution **Contributed by users** Step 18

Resuspension Buffer **Contributed by users** Step 24

Reaction Mix **Contributed by users** Step 27

Primer Mix **Contributed by users** Step 27

Positive Control **Contributed by users** Step 30

Lysis Buffer **Contributed by users** In 2 steps

Lysis Buffer **Contributed by users** In 2 steps

SAFETY WARNINGS

Biohazardous Materials

DISCLAIMER:

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.
If using the Lysis Bufferthaw the 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. Proceed to step 2.
If using the QIAmp Viral RNA Mini Kit(Qiagen cat# 52906) for lysis and RNA extraction, vortex the sample to pellet debris and start with 140 ul of input material. Proceed following the manufacturer's instructions and elute the sample in 60 ul of AVE buffer. Proceed to step 26

2 Thaw samples on ice if necessary and briefly vortex. Centrifuge the samples at 1000xg for 2 minutes to pellet solid 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. 32m

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

Create a Method on the Thermal Cycler using the following steps: 5m

10 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 should take 10-15 min, some sample types may take longer to dry, do not let drying take longer than 30 min. 15m

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).