



Dec 05, 2020

© COVID19 RTLAMP Assay_Nov_2020

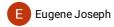
Forked from COVID19 RTLAMP Assay_Nov_2020

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¹Prime Discoveries

1 Works for me

This protocol is published without a DOI.



PROTOCOL CITATION

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FORK NOTE

FORK FROM

Forked from COVID19 RTLAMP Assay_Nov_2020, Eugene Joseph

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45274

MATERIALS TEXT

MATERIALS

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

⊠ Primer Mix Contributed by users
 Step 27

■ Lysis Buffer Contributed by users In 2 steps

SLysis Buffer Contributed by users In 2 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.
 - **If using the Lysis Buffer** thaw 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
- 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.
 - © 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

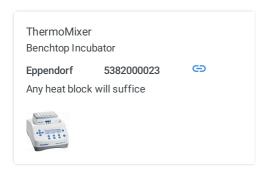
Transfer 100 μ L of the sample Input Material (or clarified supernatant if working with fresh saliva) to a new microcentrifuge tube (1.5 mL capacity) containing an equal volume of lysis buffer (e.g. for 100 μ L of sample add 100 μ L of lysis buffer). Mix the obtained solution a few times by pipetting 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



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

(900:00:30 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.



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 μ L tube containing an equal volume of lysis buffer (e.g. for 20 μ L of sample add 20 μ L 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

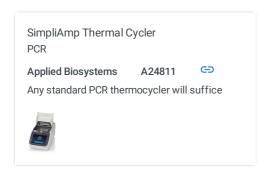
5m

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

Step 1: § 95 °C for © 00:05:00 Step 2: Hold at 8 4 °C

Lid temperature: § 105 °C

When ready run the program.



Centrifuge the tubes for 3 sec at 2000xq to spin down any condensation using a benchtop mini centrifuge. Samples are 11 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**

To each sample tube, add 800 ul of Binding Solution

■800 µl Binding Solution

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

© 00:10:00 at & Room temperature

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

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.

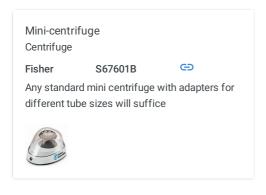


- 17 Remove the supernatant with a 1ml Pipette taking care not to disturb the pellet.
 - ■1000 µl Supernatant
- 18 Add 1ml of Wash Solution, wash the pellet by closing and inverting the tube several times.

⊠ 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



- ७ 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



@00:00:03

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

■200 µl Wash Supernatant

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.

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

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

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.

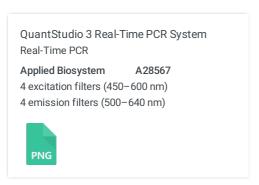
Note: As the sample contains extracted resuspended RNA, the RT-LAMP reaction should proceed the same day. The extracted samples should not be stored or frozen as it would impact the RNA integrity.



RT-LAMP REACTION

30s

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).



- 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.

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

■5 μl UltraPure Distilled Water

 $32 \quad \text{Seal the plate and run it in the Real-Time PCR instrument with the following settings:} \\$

30s

Channels and Dye

FAM - No Quencer = Assay Target Cy3 - No Quencher = Control Target

PCR STAGE

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).