COVID19 RTLAMP Assay

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¹Prime Discoveries

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Coronavirus Method Development Community  XPRIZE Rapid Covid Testing  1 more workspace

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41694
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 2 steps
- Wash Solution Contributed by users Step 17
- Resuspension Buffer Contributed by users Step 23
- Reaction Mix Contributed by users Step 26
- Primer Mix Contributed by users Step 26
- Positive Control Contributed by users Step 29
- Lysis Buffer Contributed by users In 2 steps

SAFETY WARNINGS

Biohazardous Materials

DISCLAIMER

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

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BEFORE STARTING

Heat incubator

Sample Lysis / Inactivation

1 Centrifuge the Sample in a centrifuge that can fit 15ml tubes, or let the solid debris settle for 30 min on Ice.

Thaw the Lysis Buffer on ice

2 If using a Heat Block and Following the Full Method (Includes Purification / Concentration)

Transfer 100ul of the sample Input Material (or clarified supernatant if Fresh Saliva) to a new 1ml tube containing an equal volume of 100ul of Lysis Buffer and pipette mix. Label this tube to indicate the sample name.

Lysis Buffer Contributed by users


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3 Use a tube cap lock to prevent tubes from popping open.

4 Place the tubes in a Heat block or water bath set at 95°C for 5 min

5 Carefully transfer the tubes to ice or a 4°C Cold Block for 30 sec, or let sit at Room Temperature for 5 min.

6 Centrifuge for 3 sec at 2000xg to spin down any condensation.
Samples are ready go to Step #11

7  **If using a 96 well Thermal Cycler and following the Quick Method (Without Purification / Concentration)**

Transfer 20ul of the sample Input Material (or clarified supernatant if Fresh Saliva) to a new 200ul tube containing an equal volume of 20ul of Lysis Buffer and pipette mix. Label this tube to indicate the sample name.

- **Lysis Buffer** Contributed by users
- **20 µl Lysis Buffer**
- **20 µl Clarified Sample**

8  Create a Method on the Thermal Cycler for 95°C for 5 min followed by a Hold at 4°C, with the lid set to 105°C.

- **00:05:00 95 °C**
- **Hold at 4 °C**

9  Place the tubes in the Thermal Cycler and run the method

10  Centrifuge for 3 sec at 2000xg to spin down any condensation.

- **00:00:03**


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Samples are ready go to step #25

**Sample Purification / Concentration**

11. Preheat the Heat Block or Water Bath

12. Vortex to mix the Binding Solution so there is no sediment.

13. To each sample tube, add 800ul of Binding Solution

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

15. Centrifuge for 3 sec at 2000xg to pellet the Binding Reagent in the Binding Solution Note: it should appear as a white smear at the bottom of the tube. 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.

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

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17 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**

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

- 00:00:03

- Mini-centrifuge
- Centrifuge
- Fisher S67601B
- Any standard mini centrifuge with adapters for different tube sizes will suffice

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

- **1000 µl Wash Supernatant**

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

- 00:00:03

- Mini-centrifuge
- Centrifuge
- Fisher S67601B
- Any standard mini centrifuge with adapters for different tube sizes will suffice

21 Remove the residual supernatant with a 200µl Pipette taking care not to disturb the pellet.

- **200 µl Wash Supernatant**
22. 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.

23. 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.

24. 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.

25. Prepare the qPCR instrument, have ice on hand to prepare the master Assay mix. Calculate the amount of samples being run and make sure to include the Positive Control and the H20.

26. Prepare the Assay mix by adding Reaction Mix and Primer mix according to the amount of samples being run.
12 µl Reaction Mix per Sample
3 µl Primer Mix per Sample

27 Transfer 15ul of Assay mix into the Assay wells.
15 µl Assay Mix

28 Transfer 5ul of each sample into the appropriate Assay well, pipette mix.
5 µl Sample

29 Add the Positive Control to the Plate or Strip Tube
5 µl Positive Control

30 Transfer 5ul of H20 into the appropriate Negative Control well.
5 µl H20

31 Seal the plate and run it in the qPCR instrument with the method:
Capture 2 Channels:
FAM - No Quencher = Assay Target
Cy3 - No Quencher = Control Target

00:00:30  $68.5 \, ^{\circ} \mathrm{C}$ and Capture Image
Repeat 80x