

RNA purification

1. Incubate the supernatant from step 12 at 80 degrees Celsius for 15 minutes exactly.
2. After 15 minutes, remove them and centrifuge them to bring all the droplets down to the bottom.
3. Add 320 ul of Buffer RLT and mix by vortexing.
4. Add 720 ul of 100% ethanol and mix by vortexing.
5. Transfer 700 ul of the sample into a RNeasy MinElute spin column that is placed in a 2 ml collection tube. Close the lid and centrifuge for 15 seconds at 8000 x g. Discard the flow through. Repeat this step for all the samples
6. Repeat 17 until the entire sample passes through the column.
7. Add 350 ul of Buffer FRN to the RNeasy MinElute spin column. Close the lid gently and centrifuge at 15 seconds at 8000 x g. Discard the flow through.
8. Add 500 ul of Buffer FRN to the RNeasy MinElute spin column. Close the lid gently and centrifuge at 15 seconds at 8000 x g. Discard the flow through.
9. Add 500 ul of Buffer RPE to the RNeasy MinElute spin column. Close the lid gently and centrifuge at 15 seconds at 8000 x g. Discard the flow through.
10. Add 500 ul of Buffer RPE to the RNeasy MinElute spin column. Close the lid gently and centrifuge at 15 seconds at 8000 x g. Discard the flow through and collection tube.
11. Place spin column in a new 2 ml collection tube. Open the lid and place it in the centrifuge such that the caps are facing the opposite direction of the rotation. Leave spaces in between each tube and centrifuge at full speed, (15,000 rpm) for 5 minutes to dry the column. Discard the flow through and collection tube.
12. Place the spin column in a 1.5 ml eppendorf tube and add 30 ul of RNase-free water directly on the column. Close the lid and incubate for 1 minute at room temperature. Centrifuge at full speed (15,000 rpm) for 1 minute to elute the RNA.
13. Keep the extracted RNA sample on ice if measuring OD right away, or in -80 degrees Celsius for longer storage periods.