Rneasy Prep of S2 RNA

1. Prepare fresh Buffer RLT by adding 7ul BME to 700ul Buffer RLT. (Wear gloves, work in the hood and be careful when adding the BME)
2. Pipet 10 million cells into an epindorf tube and spin in the micro centrifuge for 5 min at the lowest rpm. (be sure to balance the centrifuge for all spin steps)
3. Discard supernatant.
4. Add 600ul Buffer RLT and pipet up and down a few times to mix and homogenize the cells.
5. Add 600ul 70% ethanol and pipet up and down to mix.
6. Apply 600ul of sample to Rneasy mini column placed in a 2 ml collection tube, close the tube and centrifuge for 15 sec at 10,000 rpm. Discard the flowthrough.
7. Repeat step 6 with rest of sample in same column.
8. Add 700ul Buffer RW1to the column. Close the tube and centrifuge for 15 sec at 10,000 rpm.
9. Transfer the column to a new 2ml collection tube.
10. Apply 500ul Buffer RPE onto the column. Close the tube and centrifuge for 15 sec at 10,000 rpm. Discard the flowthrough.
11. Add another 500ul Buffer RPE to the column. Close the tube and centrifuge for 2 min at 10,000 rpm.
12. Place the column in a new 2 ml collection tube and discard the old collection tube. Centrifuge at full speed for 1 min.
13. Transfer the column to a 1.5 ml collection tube.
14. Pipet 50ul Rnase free water directly onto column membrane (without touching the membrane). Close the tube, centrifuge for 1 min at 10,000.
15. Repeat step 14 using the same collection tube.
16. Spec your RNA and store at –20.