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# MirVana RNA

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## Troubleshooting

mirVana protocol-- nasal swabs in VTM and DNA RNA ShieldDate _____

Preheat Nuclease Free water to 95C for elution
4X sets of labeled tubes (2X from the 2ml tubes provided in the kit and an additional 2X 1.5ml eppendorf tubes)

1. To **200ul** of sample add **300ul**Lysis/Binding Buffer and Vortex
2. Add **50ul**miRNA Homogenate Additive

(Hint-- Make a Master Mix of Lysis/binding Buffer and Homogenate additive)

1. Incubate on ice **10** min
2. Add **500ul**Acid-Phenol:Chloroform TAKE FROM THE BOTTOM LAYER(in the hood) Vortex for 30-60 sec
3. Centrifuge**10,000** rcf (g) for **5** min at RT
4. Remove the aqueous (upper) layer and put in a new tube (note the volume)
5. Add **1.25 volumes**100% EtOH (RT), apply to filter column with supplied tube
6. Centrifuge**10,000** rcf for **15-30** sec
7. Discard flowthrough and add **700ul** miRNA Wash Solution 1
8. Centrifuge**10,000** rcf for **15** sec
9. Discard flowthrough and add **500ul**Wash Solution ⅔
10. Centrifuge **10,000** rcf for **15** sec
11. Repeat step 11-12
12. Add **30ul** 95C Nuclease Free water on filter
13. Centrifuge **10,000** rcf for **30** sec into a supplied tube
14. Put 15ul of each sample in a labeled PCR well of a pcr tube strip and freeze at -80C