**Manual RNA Extraction of total RNA**

**Procedure**

***HOMOGENIZATION***

1. Use matrix D for tissue like kidney, liver, lung
2. Weight the tissue sample
3. 50-100 mg tissue per 1 mL TRIZOL
4. Put on ice
5. Homogenize sample ( speed 6.5, time 20sec) put on ice at least 2min

***SEPARATION***

1. Incubate 3- 5' at RT (Incubation not time critical)
2. Centrifuge Phase Lock gel Tube heavy (PLG) 2mL 1 min at 16000g
3. Transfert homogenate to Phase Lock gel Tube heavy (PLG) 2mL
4. Add 300 µL of chloroform
5. Mix on Eppendorf Thermomixer 1400 rpm 1min at RT (or Shake by hand 15'' Incubate 2-3' at RT)
6. Centrifuge at 16000g 15' at 4oC
7. Transfer the supernatant to a 2 mL tube

Break possible ( -20oC for 1-2hours, -80oC for overnight)

***Rneasy Cleanup and Dnase***

Use Rneasy Plus Mini kit , protocol Animal Tissue page 24-30, (October 2005)

1. Transfer lysate to gDNA eliminator column set on a 2mL tube
2. Centrifuge 30sec at >8000g (10000rpm) RT
3. Save the flow through
4. Add 0.6 mL 70% Ethanol and vortex
5. Apply 700 L sample to the column
6. Centrifuge 20sec at >8000g (10000rpm) RT
7. Transfer the colum to a new collection tube
8. Apply rest L sample to the column
9. Centrifuge 20sec at >8000g (10000rpm) RT
10. Transfer the colum to a new collection tube
11. Add 700 L buffer RW1 to column
12. Centrifuge 20sec at >8000g (10000rpm) RT
13. Transfer the colum to a new collection tube
14. Add 500 L RPE to the colum
15. Centrifuge 20sec at >8000g (10000rpm) RT
16. Transfer the colum to a new collection tube
17. Add 500 L RPE to the colum
18. Centrifuge 2min at >8000g (10000rpm) RT
19. Transfer the colum to a new collection tube
20. Centrifuge 1 min at full speed (14000rpm)
21. Transfer the colum to a 1.5 mL tube
22. Elute with 30-50 L Rnase free water
23. Centrifuge 1 min at > 8000g (10000rpm)