Isolation of RNA from Suspensions of Cells to NucleoPrep B

1. Pellet Cells : 100 x 10^6 - 1 x 10^7 cells into a tight pellet at 4°C at 1500 rpm for 8-10 mins. Cells can be frozen here at -80°C-140°C before proceeding.

2. Resuspend cells thoroughly on ice in 1 ml ice cold PBS and pipette up and down few times.

3. Add NucleoPrep B 200 μl/100 μl - 4°C mixture and cover tightly. Shake vigorously for 15-30 Secs (do not vortex) and let them stay on ice or 4°C for 5 mins after addition of 2 μl/20 μl lysozyme of CHCl3.

4. Centrifuge at 4°C 12,000 x g for 15 mins (9,000 rpm in 54-600: use TSO-29)

5. For larger amount of cells remove bottom organic phase and repeat Step 3 by adding 20 μl of NucleoPrep B and passing through pipette a few times. Add 2 μl CHCl3 then shake vigorously for 15 Secs, let sit on ice/4°C 5 mins.

6. Centrifuge at 4°C 12,000 x g for 15 mins (7,500 rpm in 54-600)

7. Remove aqueous phase and transfer to a fresh tube on ice and add an equal volume of isopropanol and store samples at 4°C for 15 mins or overnight here.

8. Centrifuge samples for 15 mins at 7,500 rpm at 4°C.

9. Remove supernatant and wash pellet once in 75% EtOH.

10. Centrifuge and wash pellet at 7500 rpm 4°C. Use 10 μl 75% EtOH.

11. Dry pellet briefly (DO NOT OVERDRY) by inversion on bench top.

12. Dissolve RNA pellet in 0.5% SDS in 1× EDTA pH 7. By vortexing and passing through pipette a few times.

13. Incubate at 65°C for 10-15 mins.

14. Ppt RNA in 0.2 M NaCl to one Vol. isopropanol or 2 Vols. EtOH for 1 hour at -20°C.

15. Wash pellet again in 75% EtOH: Vortex & Centrifuge at 4°C 7500 rpm for 15 mins.

16. Resuspend pellet in appropriate Vol. of RNase-free TE (H2O DEPC treated) for polyA Prep.

# Aliquot into separate tubes if not using immediately.

Store at -80°C