Isolation of mRNA from Suspension of Cells to RNAzol B

1. Pellet cells : $100 \times 10^6 - 1 \times 10^9$ cells into a tight pellet at 4°C at 1500 rpms 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
3. Add RNAzol B 2 mls / 100 mill - 500 million (pipette up and down few times) 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 mls / 20 mls lysate of $CHCl_3$
4. Centrifuge at 4°C 12,000 xg for 15 mins (9000 rpms in SA-600 : use 7500 rpms)
* 5. For larger amount of cells remove bottom organic phase and repeat step 3 by adding 20 mls of RNAzol B and passing thru pipette a few times. Add 2 mls $CHCl_3$ then Shake Vigorously for 15 secs. let Sit on ice/4°C 5 mins.
6. Centrifuge at 4°C 12,000 xg for 15 mins (7,500 rpms in SA-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 rpms at 4°C.
9. Remove Supernatant and Wash pellet once in 75% EtOH by Vortexing and Centrifugation at 7500 rpms 4°C. Use 10 mls 75% EtOH.
10. Dry pellet briefly (* DO NOT OVERDRY) by inversion on bench top.
11. Dissolve the RNA pellet in 0.5% SDS in 1 mM EDTA pH 7. by Vortexing and passing thru' pipette a few times.
12. Incubate at 65°C for 10-15 mins
13. Ppt. RNA in 0.2 M NaCl w one Vol. isopropanol / or 2 vols. EtOH for 1 hour at -20°C.
14. Wash pellet again in 10 mls 75% EtOH : Vortex & Centrifuge at 4°C, 7,500 rpms for 15 mins.
15. Resuspend pellet in appropriate Vol. of RNase free TE (H_2O DEPC treated * for poly A prep.)
16. Take Spec. Reading for Concentration.

* Aliquot into separate tubes if not using immediately.
Store at -80°C