

Preparation of RNA from B Lymphocytes

1. Harvest approx. 50mls of Culture and centrifuge to collect cells at 4°C for 5 mins at 1000rpm.
2. Resuspend cells in 250 μl 1x PBS gently to prevent lysis and add 10mls of RNazolB pipetting up and down to effectively lyse cells. Transfer to Corax tubes on ice.
3. Add 1ml of CHCl_3 and using a double folded parafilm shake the 30ml Corax tubes vigorously for 15-30 secs. Let tubes sit on ice for 5 mins.
4. Centrifuge in Beckman swinging bucket JS13 rotor at 8000rpm for 15 mins.
5. Remove the clear upper aqueous layer (approx. 4-5mls) to a fresh prechilled Corax tube and add 5mls of Isopropanol. Store at 4°C for 15 mins.
6. Centrifuge at 8000rpm for 15 mins to collect RNA and resuspend in 2ml DEPC treated water. Add 2mls of Isopropanol and centrifuge after 15 mins on ice for 8000rpm at 4°C for 15 mins.
7. Add 10mls of RNase free 75% Ethanol and centrifuge at 6,000rpm for 10 mins.
8. Air dry pellet and resuspend in 60 μl RNase free TE buffer. Do not let RNA dry completely.
9. Take optical density. Calculate Concentration.
Load approx. 10 μg RNA per well up to 30 μg