



Cryopreservation of cells

Materials and Equipment

1. Human PBMCs, BMMNCs, Fixed WB, tissue samples or Mouse cell suspension
2. 10% DMSO (Sigma #D4540-100ML) + 90% FBS (VWR #HYCLSV30180.03)
3. Cell counter (Biorad)
4. Cryovials (VWR # 89094-802)
5. Mr.Frosty (VWR #55710-200) with Isopropanol
6. -80°C Freezer or -150°C Freezer or Liq Nitrogen

Procedure

1. Label cryovials to freeze the entire sample. Label should include sample identification name and date
2. Count cells using the ViCell (or Hemocytometer with viable cell exclusion dye) and take required amount of cells (RBC's lysed) to be frozen by resuspending them in FBS
3. Adjust the cell concentration to 5-10 X 10⁶ cells/ml/vial using FBS at RT
4. Prepare freezing medium with 10% DMSO + 90% FBS (freshly prepare this every time by calculating the amount of medium needed with 0.5-1ml/vial)
5. Spin the tube at 300g for 5 min with adjusted cell concentration above and add the same amount of freezing medium to the cell pellet
6. Slowly remove the cell suspension into a pipette and dispense 0.5-1ml per cryovial
7. Place the cryovials at RT in Mr. Frosty freezing container
8. Place the freezing container as soon as possible into the -80°C freezer
9. Transfer the cryovials to liquid nitrogen tank or -150°C after 24 hrs