



### **SOP 1.1 – Cryopreservation of cells**

#### **Materials and Equipment**

1. Human PBMCs or Mouse cell suspension
2. 10% DMSO (Sigma #D4540-100ML) + 90% FBS (VWR #HYCLSV30180.03)
3. Hemocytometer or ViCell counter
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, date, and 'PBMC'
2. Count cells using the ViCell (or Hemocytometer with viable cell exclusion dye) and take required amount of PBMCs or Splenocytes (RBC's lysed) to be frozen by resuspending them in FBS
3. Adjust the cell concentration to  $5-10 \times 10^6$  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 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 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