

Reagents/Materials

Reagent	Vendor	Catalogue #
DMSO	Roth	A994.1
Fetal Bovine Serum (FBS)	Biochrom	S 0115
Internal threaded Cryotubes	Oehmen	122 263
Cryo Labels	LabID	N0A4CL-8T1-WH
Mr. Frosty freezing container	Oehmen	9.400 945

Freezing Cells

1. Determine the number of live cells being frozen by either counting the cells. Determine the number of internal threaded cryo vials needed. Note: we usually freeze 10-100 x 10⁶ PBMCs or 5 x 10⁶ clones per vial.
2. Using the computer and label template file, create labels with the appropriate number of internal thread cryo vials (alternatively you can write the information on the tube by hand):

HIV+	HIV-
Patient ID number	Patient ID Number
Patient Case number	10-100 x 10 ⁶ PBMCs
10 x 10 ⁶ PBMCs	Today's Date
Today's Date	

3. Place the labels on the cryo vials and return them to the hood
4. Prepare a solution of 20% DMSO in FCS and put on ice to pre-cool.
5. Spin cells to be frozen using Program 4 (4°C cold centrifuge, 10min, 500xg).
6. During the spin, calculate the volume of FCS to resuspend the cells in and the total volume of 20% DMSO/FCS to make (these 2 volumes are equal). An easy way to do this is multiply the number of vials you want to freeze the cells in by 1.5 (since we freeze them in 1.5mL) and divide by 2. For example:

Patient	# vials to freeze	Multiply by 1.5	Divide by 2 - Volume FCS (ml) to resuspend cells
A	6	9	4.5
B	8	12	6
C	10	15	7.5
D	12	18	9
Total volume 20% DMSO/FCS to make rounded up to nearest multiple of 5			4.5+6+7.5+9 = 27 → 30 ml

7. After the spin, aspirate tube to about 200 µl and resuspend the pellet in the calculated amount of cold FCS for each patient. Place cells on ice.
8. Add equal volume of 20% DMSO/FCS to resuspended cells, drop-wise, while shaking the conical of cells to mix. Final freezing solution is 10% DMSO/FCS
9. Dispense 1.5 mL of resuspended cells in each vial. Place aliquots on ice while aliquoting other patients.
10. Do not keep the vials containing cells and freezing solution on ice for too long before they are placed in the -80° freezer. DMSO is toxic to cells, so their viability will suffer if they are not frozen quickly enough. Don't freeze too many simultaneously if you lack experience.
11. Place vials in a Mr. Frosty that was pre-cooled to 4°C (in the refrigerator). Make a line on the label on top of the Mr. Frosty (each line = one use) and write your initials under the mark.
Note: If the label already has 5 marks on it, then empty the isopropanol into the sink and refill it with 250ml fresh isopropanol found in the safety drawer under the fume hood. Place this newly filled one in the 4°C to cool and retrieve a new, chilled Mr. Frosty.
12. Place the Mr. Frosty(s) in the -80°C.
13. Cells are transferred to liquid N₂ after on the following day. **You are responsible for transferring anything you have frozen to the Liq N₂!!** Please make sure cells are placed in the appropriate rack/box. Record the locations of each vial on a post-it note and be sure to update the Liq N₂ log on the server.

It is incredibly important that cells are transferred to the Liq N₂ by the next day as they can decrease in viability if left in the -80°C (due to the frequent door opening). The person who froze the cells is responsible for transferring them the next day, or for asking someone to transfer them. Anyone who's caught with a Mr. Frosty in the -80°C after one day will take over processing all blood (HIV+ and HIV-) for one full work week or you may pay 1€ per day you do not/cannot process the blood (max 5€).