

Institut für HIV Forschung

Reagents

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	Thermo	5100-0001

Freezing Cells

1. Determine the number of live cells being frozen by either counting the cells, or if they were counted that day, check the logbook. Determine the number of internal threaded cryo vials needed. Note: we usually freeze 10×10^6 PBMCs or 5×10^6 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):

Patient ID number
Patient Case number
 10×10^6 PBMCs
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° cold centrifuge, 10min, 600xg).
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			$4.5+6+7.5+9 = 27 \rightarrow 30 \text{ ml}$

7. After the spin, aspirate tube to about 200 μ l 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 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) and place container and cells in -80°C .
12. Cells are transferred to liquid N_2 after 4hr or next day if frozen in the afternoon.