Institut für HIV Forschung
SOP #08-00 (BS - **Juli 2016**)

Thawing Cells

Reagents

Reagent	Vendor	Catalogue #
DMSO	Sigma	2650
RPMI-1640 media	Life Technologies	31870-074
L-Glutamine	Sigma	59202C-100ML
Pen/Strep (5000IU Pen/5000ug/ml strep)	Sigma	0781
FBS	Biochrom	S 0115

Media

R10:

Reagent	Stock Concentration	Volume to Add	Final Concentration
RPMI 1640	-	500	-
FBS	100% (v/v)	55ml	10% (v/v)
Penicillin/Strep	Pen: 5000 IU/mL Strep: 5000ug/mL	5.5mL	Pen: 50 IU/mL Strep: 50ug/mL
L-glutamine	200mM	5.5mL	2mM
HEPES	1M	5.5ml	10mM

*Cells are frozen in 10% DMSO in FCS.

To thaw warm: Pre-warm R10 to 37° in the water bath and spin at room temp (25°).

1. Pre-label 15 mL conicals (one for each vial you thaw), and add 9mL of R10. Leave them in the hood.
    a. For vials containing more than $20x10^6$ cells, use a 50mL conical with a larger volume of R10. A good rule of thumb is cells should not be a higher concentration than $2x10^6$/mL
2. Thaw cryopreserves tube with cells in 37° water bath until there is a small pellet of ice visible. **Don't** wait until the entire vial is thawed! Once you see the vial starting to thaw, wipe it with alcohol and transfer it into the hood. Ideally, you want the cells thawed for as little time as possible before they are diluted in R10 and spun down so that the DMSO has little time to harm them. (DMSO is toxic to cells.)
3. Wipe the vial with alcohol and then transfer the vial into the hood. Transfer the cells into the 15 ml conical tube using a 5mL pipette.
4. Using 1ml R10 from the conical, wash out cryo-vial to get the remaining cells.

5. Spin cells for 10 minutes in centrifuge at 300xg.
6. Aspirate off as much of the freezing solution as possible without touching the pellet.
7. Resuspend in 10 ml of R10 medium for counting. Take a small aliquot and place into a clean eppendorf tube.
8. Spin again for 10 minutes at 300xg.
9. While cells are in the second spin, count the cells from the aliquot.

BCL

After the second wash, aspirate off the media and add 5ml R20. Transfer into a T25 and place in the incubator.

CTLs

Have feeders solution ready (irradiated allogeneic PBMC in R10). After second wash, transfer cells to T25 upright with 20 million feeders /20 ml volume and anti-CD3 (12F6) at 0.1 μg/ml in R10/50. Then feed twice a week according to the regular schedule.
Remember to always make fresh medium and fresh made feeders for thawing CTLs.

PBMCs

For ELIspot we thaw in R10 (to decrease background).