Institut für HIV Forschung
SOP #02-03 **(September 2015 - BS)**

T Cell Line Generation

Reagents

Reagent	Vendor	Catalogue #
RPMI-1640	Apotheke	31870-074
L-Glutamine	Apotheke	M 11-004
Pen/Strep	Apotheke	P 0781
Dynabeads® Human T-Activator CD3/CD28 for T Cell Expansion and Activation	Thermo	11131D
rhIL-7	R&D Systems	207-IL-025
rhIL-2	R&D Systems	202-IL-050
AZT		

****All work must be done in a sterile hood. The hood should be cleaned and UV light turned on for >1hr prior to any work being performed****

Day 0

- Thaw PBMCs according to SOP 08-00
- Let PBMCs rest 4hr or overnight in R10
- Deplete CD8 using positive isolation according to SOP 23-00
- Resuspend remaining cells in R10 (or RAB10) and count
- Spin down cells and resuspend to 1-2 million cells/ml
- Plate cells
    - Please note that cell lines in wells along the edge of the plate tend to not grow well. Best thing to do is use only wells in the middle and fill wells along the edge with water or PBS
    - For 24-well plates use 2ml of cells and for 6-well plates use 4ml of cells
- Add stimulation factors
    - For nonspecific stimulation use anti-CD3 and anti-CD28 beads
    - For antigen specific stimulation us 1µg/ml of Gag or Env PTE pooled peptides
    - For peptide specific stimulation use 10µg/ml Gag or Env Con B peptides
- Add 25ng/mL rhIL-7 (R&D Systems cat# 207-IL-025, -20C TC lab)
    - 25µg of IL-7 is dissolved in 500µl of PBS for a final concentration of 50µg/ml therefore use **0.5µl/ml**
- Add 2µg/ml AZT (Sigma cat# A2169-25mg, -20C TC lab)
    - 25mg of AZT is dissolved in 12.5ml PBS for a final concentration of 2µg/ml therefore use **1µl/ml**
- Mix wells thoroughly using a P1000

- Place in a 37C incubator that is not used often to prevent humidity and temperature changes. Disturbances such as these can hinder cell growth.

Day 2/3

- Add 1800U/ml IL-2 to each well
    - Working stock of IL-2 is kept at $1x10^5$ U/ml in PBS therefore use **18µl/ml**
- Mix wells thoroughly using a P1000

Day 7

- Supplement wells with fresh, warm media
    - When transporting cells to hood be careful to not disturb the cells as they are all settled at the bottom of the well. Exchange media by carefully aspirating half the well's volume with a P1000 pipette and replacing it with fresh media.
- Add 1800 U/ml IL-2
- Check the confluency of cells and if necessary split cells into a new well or freeze them down in 10%DMSO/90%FCS for future use.

Day 10

- Thoroughly resuspend cells using a P1000 and count.
- If there are enough cells, remove a portion for experiments but leave >30-50% to continue cell lines whenever possible. Leftover cells should have media replaced with fresh, warm media and add 100 U/ml IL-2

From this point on cells should have 100 U/ml IL-2 added twice weekly with a media refresh once a week. AZT can be added with IL-2 if desired at 2ng/ml. Restimulation should only be performed when necessary and cells should be counted with every media refresh or with IL-2 addition to monitor health. In the event of rapid cell proliferation in which cell numbers $>2x10^6$/ml, cells should be split into a new well or frozen down as described above. Upon cell number decline (which can happen within 1-2 months) cells should be used immediately for any desired experiments.