Institut für HIV Forschung
SOP#02-04 **(Oktober 2016 - ER)**

HIV peptide-specific CD4 clones

Preparing of feeder cells

- The day before sort thaw cells from two different donors
  - In total 200 000 cells/well from the two different donors
  - 100 000 cells/well from each donor
  - 10M cells/plate from each donor for a 96-well plate
- Let the cells rest over night
- The next day deplete CD8 using positive isolation according to SOP 23-00
- Resuspend remaining cells in or RAB10 and count
- Spin down cells and resuspend to 1 million cells/ml → 10M cells in 10ml from each donor for one 96-well plate
- Put the cells separately in small petri dishes and irradiate them at 30gray
- Afterwards collect cells separately in falcon tubes and add 10U/ml IL-2 and 5µg/ml PHA to the cells
- Plate cells before sort
  - 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
  - Use 96-well U/round bottom-plates and add 100µl cell suspension from donor A and 100µl cell suspension from donor B to the wells. In total you should have 200 000 cells/well

Stimulation and Interferon-gamma capture assay

- First thing you have to do is depleted CD8s (depletion Kit from Miltenyi)
- Stimulate the cells in FACES tubes with 1ml of the cell solution overnight or 18h with 14-20µg/ml Peptide
- On the next day perform the Interferon-gamma capture assay (IFN-gamma secretion Assay, Detection Kit APC from Miltenyi)
  - To reduce the background it is possible to use only the half of the recommended amount of the catch reagent
  - Think about to use 3ml for the secretion period
  - During secretion period rotate for 1 ½ hours
- Afterwards stain the cells (also include Live/Dead staining) and prepare the compensation beads
- Do not forget to prepare the 96-well U/round bottom-plates

Sort

- Set the gates for your Interferon-gamma+, CD4+ cells according to your positive and negative control
- Keep the plates at 37 °C (when you have the possibility)
- Sort the single-cells and keep one plate as a control plate with only feeder cells

Feeding

- Put the cells in an incubator without traffic
- Don't touch (not even a look at) the cells for at least 10 days
- After 10 days check if the media in some wells turning orange/yellow (probably these are your clones)
- Let them rest for another 4 days
    - Check the wells which are turning orange/yellow under the microscope → the cells should clump together
    - Change the media for these wells (Exchange media by carefully aspirating half the well's volume and replacing it with fresh media containing 10U/ml IL-2 → because of the dilution you have to double the amount of IL-2)
    - Split then the positive wells in 48-well plates
- Feed them twice a week and check under the microscope for clump-formation
- Split the cells in 24-well plates when the clones are growing well in the 48-well plate (some clones will grow faster/better than other)
    - Check now also for the viability
- Split the cells again in 12-well plates after some time
    - Think about to freeze some cells