Institut für HIV Forschung
SOP #02-01 (April 2015)

Cloning of peptide-stimulated PBMCs

1. Make fresh R10/50 for cloning before starting procedure. See Protocol #3 Media Preparation.
2. Dilute effector cells to 100,000 cells/ml (.1M/ml) to be cloned.
3. Take out the correct number of sterile 96 well culture plates. Open them inside the hood only to preserve their sterility.
4. One plate will contain the following:
    a. 10 million irradiated allogeneic feeders.
    b. Anti-CD3 mAb. You can use either 0.1 µg/ml of 12F6 or 0.25 µg/ml of PHA. Do not add stimulator more than 10 minutes before seeding cells on the plate.
    c. Effectors—either 30 µl, 10 µl, or 3 µl depending on the chosen dilution.
    d. 20 ml of fresh R10/50.

For 30 cells/well:
Total volume on the plate is 200 µl/well x 96 wells = .2 ml.
To convert the solution to cells/ml, 30 cells/well x 5 = 150 cells/ml
1 plate = 100 wells x 200 µl = 20 ml
x (100,000 cells/ml) = 20 ml (150 cells/ml)
x = 30 µl

For 10 cells/well:
10 µl of 100,000 effector cells/ml to be cloned in 20 ml of feeders solution.

For 3 cells/well:
Use 3 µl of 100,000 effector cells/ml to be cloned in 20 ml of feeders solution.

5. Place 200 µl in each well. Do not feed for 6-7 days. After that, exchange R10 medium (100 µl out, 100 µl in). Make sure not to disturb cells at the bottom when feeding.
6. After 14 days in culture, single wells will start to grow (15-35% at 30 cells/well, 12-18% at 10 cells/well).
7. Keep feeding for 21 days. After that, transfer growing wells into a 24 well plate with 1 million irradiated allogeneic feeders per well in R10/50 and anti-CD3.
8. Culture it for 14 days and test for cytolytic activity in Cr51 assay. See Protocol #14.
9. **Note**: if you only need to test one peptide, then the Cr assay can be done directly from the 96 well plates. Only 2 targets need to be checked—blank (no peptide) and the chosen peptide. In this case, take 25 µl of the effector suspension from the 96 well plate for each target. Add medium back onto plate afterwards.
10. If you need to check more than 2 peptide targets, then the cells need to be expanded first.

Freshly isolated PBMCs can also be cloned at 30, 10, or 3 cells/well with non-specific stimulator only (Anti-CD3 or PHA); the procedure for cloning remains the same. After culture, growing wells must be expanded in a 24 well plate and tested with a recombinant vaccinia virus expressing all HIV proteins as well as a control. We use autologous BCL lines infected with

vaccinia for 16 hours. Positive wells must then be expanded and screened again with smaller vaccinia pools and mapped with peptide to determine epitope sequence.