Creighton UNIVERSITY

Mixed Lymphocyte Cultures

(Greg A. Perry, Ph.D.; *after Robinson et al. (1976?)*)

Equipment:

Round Bottom Microtiter Plate (Costar #3799)
Gamma-Irradiator (^{137}Cs source)
Glass Fiber Filters (Brandel, or Whatman #1827-887)
PhD cell harvester (Brandel, Model 290)
Scintillation Counting Vials (Fisher #03-337-1 or Research Products Int'l #125516)

Reagents:

RF10-M Media (Sterile)
Cell Preparations (Sterile)
- Responding cells: @ $2x10^6$ cells/ml in RF10-M media.
- Stimulating cells: @ $5x10^7$ cells/ml in RF10-M media.

^{3}H-Thymidine (Amersham)
Scintillation Counting Fluid (PCS, Amersham #NPCS104 or Bio-Safe NA, Research Products Int'l #111198)

Method:

1. Stimulating cells must be irradiated with 2500 rads of ^{137}Cs irradiation immediately prior to use.
2. Add 100 µl of responding cells to each appropriate well of the microtiter plate. ($2x10^5$ cells per well)
3. Add 100 µl of stimulating cells (or media) to each appropriate well containing cells. ($5x10^6$ cells per well; producing a stimulator/responder ratio of 25:1)
4. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air for 78 hours.
5. Dilute thymidine to a concentration of 20µCi/ml in RF10-M.
6. Add 50 µl of diluted thymidine to each well (1 µCi/well).
7. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air for an additional 18 hours.
8. Harvest cells onto glass fiber filters using a cell harvester.
9. Allow filters to completely air-dry overnight on aluminum foil.
10. Place filter disks into liquid scintillation bags and add 2ml PCS liquid scintillation fluid.
11. Count in a liquid scintillation counter.
12. Express results as either ΔCPM or as Stimulation Index (SI) as calculated below.

ΔCPM: ΔCPM is calculated as …

ΔCPM = ($Mean_{CPM}$ of mitogen stimulated wells) - ($Mean_{CPM}$ of media stimulated wells)

For example, if the mean CPM of the stimulated wells is 2350, and the mean CPM of the media stimulated wells is 124, then the ΔCPM = 2350 – 124 = 2226.

Stimulation Index (SI): Stimulation index is calculated as …

SI = ($Mean_{CPM}$ of mitogen stimulated wells) / ($Mean_{CPM}$ of media stimulated wells)

If we use the same values as above, then SI = 2350 / 124 = 18.95