Creighton
UNIVERSITY

Mitogen Assay in Microtiter Plates

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

Equipment:

Flat Bottom Microtiter Plate (Costar #3596)
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 Preparation (Steriile)
- at $2x10^6$ cells/ml in RF10-M media

Mitogens (pre-diluted)
- Concanavalin A (Con-A) (Type IV; Sigma #C-2010)
- Phytohemaglutinin-A (PHA) (Type M; Gibco #10576-015 or Sigma #L-8902)
- Lipopolysaccharide (LPS) (Serotype 055:B5; Sigma #L-2880)

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

Method:

1. Add 100 µl of cells to each appropriate well of the microtiter plate. ($2x10^5$ cells per well)
2. Add 100 µl of diluted mitogen (or media) to each appropriate well containing cells.
3. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air for 52 hours.
4. Dilute thymidine to a concentration of 20µCi/ml in RF10-M.
5. Add 50 µl of diluted thymidine to each well (1 µCi/well).
6. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air for another 15-18 hours.
7. Harvest cells onto glass fiber filters using a cell harvester.
8. Allow filters to air dry overnight on aluminum foil.
9. Place filter disks into liquid scintillation bags and add 2ml PCS liquid scintillation fluid.
10. Count in a liquid scintillation counter.
11. Express results as either ΔCPM or as Stimulation Index (SI) as calculated below.

Note: Each new batch of mitogen must be tittered to determine optimal concentration for stimulation. Typical concentrations would be:

Con-A: ~ 5 µg/ml (1-5µg/ml)
PHA: ~ 5 µg/ml (1-5µg/ml)
LPS: ~ 40 µg/ml (2-50µg/ml)

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

Reference: *Robinson JH and JJT Owen, Generation of T-cell function in organ culture of foetal mouse thymus: I. Mitogen responsiveness., Clin. Exp. Immunol. 23:347-354, 1976.*