Creighton UNIVERSITY

MTT Mitogen Assay in Microtiter Plates

(Greg A. Perry, Ph.D.; *after Mossman (1983)*)

Equipment:

Flat Bottom Microtiter Plate (Costar #3596)
Dual beam ELISA reader

Reagents:

RF10-M Media (Sterile)
Cell Preparation (Steriile)
- at $5x10^6$ 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)

MTT Stock (5 mg/ml in PBS; keep in dark at 4^oC. Stable for several weeks.)
Acid-Alcohol (0.04N HCl in 2-propanol)

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 mitogen (or media) to each appropriate well containing cells.
3. Incubate at 37^oC in a humidified atmosphere of 5% CO_2 in air for 72 hours.
4. Resuspend cells in each well, and transfer 25 µl of cells from each well into a corresponding well on a new plate containing 75 µl of fresh RF10-M in each well.
5. Add 10 µl of MTT stock to each well and mix by tapping gently on the side of the tray.
6. Incubate for 4 hours at 37^oC for cleavage of MTT to occur (optimal time may vary).
7. Add 100 µl of acid-alcohol to each well and mix by pipetting up and down several times.
8. Within an hour, measure the absorbance on an ELISA plate reader with a test wavelength of 570 nm and a reference wavelength of 630 nm.
9. Express results as Optical Density (OD).

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

Con-A (Sigma, Type IV):	~ 5 µg/ml
PHA (Wellcome):	~ 5 µg/ml
LPS (Sigma, Serotype #055:B5):	~ 40 µg/ml

Reference: *Mossman, T. (1983) Rapid calorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assays. Journal of Immunological Methods 65: 55-63.*