

Mitogen Stimulation in Bulk Cultures

(Greg Perry, Ph.D.)

Equipment:

Tissue Culture Flask (25cm², Corning #430168) or Tube (Falcon #352059)

Reagents:

RF10-M Media (Sterile)

Cell Preparation (Sterile)

- at 2x10⁶ cells/ml in RF10-M media

Mitogens (pre-diluted)

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

Method:

1. Add 5ml of cells to each appropriate T-flask. (1x10⁷ cells per flask.)
 - If using tubes, add 2ml of cells to each tube.
2. Add 5 ml (or 2ml) of diluted mitogen (or media) to each appropriate flask (or tube) containing cells.
3. Incubate at 37°C in a humidified atmosphere of 5% CO₂ in air for 48-72 hours.

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)