

MITOGEN STIMULATION

PHA*P: Burroughs-Wellcome #HA16, 2 mg vials

Titer each batch to determine optimal concentration for stimulation.

Con-A: Miles Labs #79-003, 250 mg bottle lyophilized, \$22.00

Tritiated Thymidine: Thymidine (methyl-3H) #2533-96 Schwarz-Mann,
5.0 mCi in 5.0 ml (sp. act. 6Ci/mMole) Request sterile aqueous vs. 2% Ethanol.

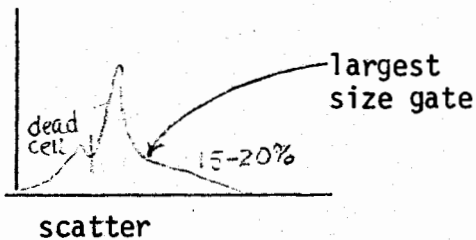
1. Mitogen Stimulation:

Take thymuses out sterilely into D's PBS + pen strep

Geys to remove red cells

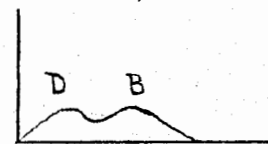
Glass wool to remove dead cells

Stain 20' at RT 1:20 α - θ



take lg - bright small - bright
lg - dull small - dull

Scatter gate for largest 15% + smallest 15%
Then separate for Bright and Dull



Scatter Gated Fluorescence
of largest 15-20%

Culture:

5×10^5 cells in 200 μ l/well with 1 μ l either PHA* or conA* in 20 μ l - in triplicate.

	conA	PHA	PBS
Unstained whole			
Stained whole			
LB			
LD			
SB			
SD			
Mix LB + LD + SB + SD			
	↓	↓	↓

*titer each batch

2. Assay

1. On day 3, pulse with 1 μC H^3 thymidine/well in 10 λ .
2. 4 hours later, ppt protein with 20 λ of 50% TCA @ room temperature/well.
3. Centrifuge @ 2000 rpm and wash 3 x with 5% TCA @ room temperature. Resuspend with microtiter mixer on speed setting #3.
4. Add 100 λ of 1.0N NaOH to pellet and mix 20-30 minutes (or let stand overnight @ 4°C).
5. Add dissolved sample to 10ml aqueous scintillation fluid and count in β counter. 1 well/10 ml vial.