

MIXED LYMPHOCYTE REACTION

Will need:

- a. Stimulating cells (mitomycin treated or irradiated or F₁ H2 type so as not to show DNA incorporation of ³H-thymidine in culture), and
- b. Responder cells (parental cells if H2 of F₁ is sensitizing Ag).

1. Stimulating Cells

a. Whole spleen

Take spleens out sterilely into D's PBS + pen strep (~6) Gey's, but omit the wash and the FCS.

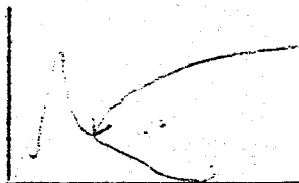
Pass through glass wool. Elute immediately. Use a fairly rapid flow rate.

b. T-cell preparation from whole spleen

1. Use nylon wool column with sterile technique to isolate T, or
2. Stain whole spleen with $\alpha \theta$ and use cell separator to obtain T-cells.

Stain 10^7 cells per 100 λ of Fl. $\alpha \theta$ @ 1:20.

Stain for 20 minutes @ room temperature.



gate here so that to the right = 50%
(44% = average % splenic T cells).
Because very brights = dead, so effective
recovery = 44% T-cells live.

Scatter gated fluorescence

Then split the 50% stained with $\alpha \theta$ into 22% Bright + 22% Dull, leaving a 6% window between to avoid overlap.

Collect $\sim 10 \times 10^6$ of B and D cells.

Resuspend in RPMI 1640 + pen strep 50 ug/ml

Adjust to $5 \times 10^5/100 \lambda = 5 \times 10^6/\text{ml}$ (or, if short on cells, bring up to necessary volume and count)

Range is $25 \times 10^5 - 5.0 \times 10^5/100 \lambda$

Number of Cells Needed for MFR

5×10^5 cells/well cultured										
Cell Type	Controls	Mixture	Experimentals	TOTAL						
F ₁	$2.5 \times 10^5 \times 2 \times 3$	—	$2.5 \times 10^5 \times 15$	5.25×10^6						
✓ Parental in hole unstained	$2.5 \times 10^5 \times 2 \times 3$	—	$2.5 \times 10^5 \times 3$	2.25×10^6						
Parental T stained	$2.5 \times 10^5 \times 2 \times 3$		$2.5 \times 10^5 \times 3$	2.25×10^6						
Parental B cells	$2.5 \times 10^5 \times 2 \times 3$	$1.25 \times 2 \times 3 \times 10^5$ $1.25 \times 10^5 \times 3$	$2.5 \times 10^5 \times 3$	3.4×10^6						
Parental D cells	$2.5 \times 10^5 \times 2 \times 3$	$1.25 \times 2 \times 3 \times 10^5$ $1.25 \times 10^5 \times 3$	$2.5 \times 10^5 \times 3$	3.4×10^6						
<p>To separate B + D cells totaling 7×10^6 cells, need 4×10^7 cells put on the machine.</p>										
<p>Approx @ $5000 \text{ cells/cc} \times 3600 \text{ cc} = 18 \times 10^6 \text{ cells/}$ $\frac{18 \times 10^6}{4} = 4.5 \times 10^6$</p>										

2. Responder Cells

2 spleens sterilely into RPMI 1640 + pen strep + glutamine

Gey's - as above

glass wool

Adjust to $5 \times 10^5 / 100 \lambda = 5 \times 10^6 / \text{ml}$

3. Culture

set up cultures in rigid "V" microtitre plates using 100λ of each cell type per well* (200λ total well volume).

controls = 1. sensitizer x sensitizer

$(F_1 \times F_1)$

2. whole unstained responder spleen x itself

$(WUS \times WUS)$

3. whole stained responder T cells x itself

$(WST \times WST)$

4. whole unstained responder T cells x itself

$(WUST \times WUST)$

5. Bright T x Bright T

$(B \times B)$

6. Dull T x Dull T

$(D \times D)$

7. mix of B+D x mix of B+D

$(B+D \times B+D)$

experimentals

each of 6 responder cell types cultured with F_1 cells.

*NOTE: Use centermost microtiter wells because their contents mix better on the microtiter mixer, and are more reliably exposed to the same surface gas conditions.

4. Assay

3 days later:

Pulse with $1 \mu\text{C } H^3$ thymidine in 10λ ~~(1 $\mu\text{C}/\text{ml}$)~~

4 hours later: add 20λ 50% TCA/well (bring TCA to room temperature)

Centrifuge for 10 minutes at fastest speed of IEC using microtitre plate carriers + head.

Flick to discard supernatant, drain microtitre plate

Wash x 3 with 5% TCA at room temperature, resuspending on shaker (setting #3)

Add 100λ 1N NaOH

Shake 20' - 30' or let sit overnight in cold room until dissolved.

Transfer NaOH into 10 ml scintillation fluid for aqueous samples and refrigerate to count in β counter (Use a pulled pasteur pipet)

(see liquid scintillation counting protocol)