

SEPA  
ABSORPTION OF ANTIBODY WITH LYMPHOID CELLS

\*\*\*\*\* Inactivate sera before absorption \*\*\*\*\*

1. Thymus cells as absorbant

- a. 2 mouse (age 4-6 weeks) thymuses  $\approx 2 \times 10^8$  (excess) / 10 mg protein to be absorbed. Bone marrow  $6 \times 10^7$  cells / 10 mg protein.
- b. Pellet cells well @ 12-1500 rpm for 15 minutes after washing 2x in PBS, and discard all supernatant.
- c. Resuspend cells in protein solution to be absorbed and leave in ice for one hour. Resuspend a few times during the hour.
- d. Centrifuge @ 1200 rpm for 15 <sup>Minutes.</sup>
- e. Ultracentrifuge supernatant for 2-1/2 hours at 100,000g ~~rpm~~.  $\sim 40K$  rpm
- f. Sterilize supernatant by millipore filtration.

$\frac{10^8 \text{ Thymus}}{2 \times 10^5 \text{ Spleen}} = 5 \times 10^7 \text{ MLN}$   
 $\frac{3 \times 10^8 \text{ cells}}{\text{mouse}} = 10 \text{ mg protein}$

To be Absorbed	B.M.	Lymph	Mouse Liver	Thymus	SRBC
Rabbit S		$\frac{2 \text{ mice}}{1 \text{ ml inact. sera}}$		$\frac{2 \times 10^8 \text{ T}}{10 \text{ mg}}$	
FCS		$\frac{1 \text{ mouse}}{10 \text{ ml FCS}}$	$\frac{1 \text{ gm liver}}{100 \text{ ml inact.}}$		
C <sup>1</sup>					$\frac{1 \text{ ml packed SRBC}}{1 \text{ ml C}^1}$
R <sup>2</sup> T	$\frac{3 \text{ mice}}{3 (2 \times 10^7 \text{ bmc/ mouse})}$				
	ml				

At least  $1.5 \times 10^7$  PBL cells / 5 mg protein or equal volume packed cells / neat sera.

Expected Yield

$2 \times 10^7$  bmc / mouse  
 $1 - 2 \times 10^8$  thymus / mouse  
 $5 \times 10^7$  lymph nodes + mesenteric