

## 1° Splenic B Cell Isolation

Day -1:

1. Plate  $7.1 \times 10^6$  irradiated CD-40 L cells in a T162 flask in 20 mls L cell medium.

L cell Medium  
DMEM+  
10% FBS  
L-glutamine  
2-Mercaptoethanol  
Plasmocin

Day 0:

2. Collect spleen.
3. Crush half of spleen between two frosted glass slides and wash through nytex filter in 10 mls medium (preferably RPMI with at least 5% FBS).
4. Centifuge and decant.
5. Resuspend in 5 mls ACK and incubate at 37°C for 5 min.
6. Centrifuge and decant (should have  $\sim 40 \times 10^6$  cells/mouse).
7. Resuspend in 40 mls 1° Splenic B cell medium/mouse.

1° Splenic B cell Medium  
RPMI+  
10% FBS  
L-glutamine  
2-mercaptoethanol  
Pen/Strep  
Cyclosporin A ( $5.5 \times 10^{-8}$ M)  
10 pg/ml mIL-4 (from RDI)

8. Pour off medium from L cell flasks and add all splenocytes from one mouse to a flask in the 40 mls.

Day 2:

9. Plate  $7.1 \times 10^6$  irradiated CD-40 L cells in a T162 flask in 20 mls L cell medium.

Day 3:

10. Pour medium off L cells.
11. Collect splenocytes from each flask, count, centrifuge and decant.
12. Resuspend splenocytes in 1° Splenic B cell Medium and plate  $40 \times 10^6$  cells/ flask in 40 mls 1° Splenic B cell Medium. (Be careful not to put too many splenocytes/flask or the L cells wil die)

Day 6:

13. Plate  $7.1 \times 10^6$  irradiated CD-40 L cells in a T162 flask in 20 mls L cell medium.

Day 7:

14. Pour medium off L cells.

15. Collect splenocytes from each flask, count, centrifuge and decant.

16. Resuspend splenocytes in 1° Splenic B cell Medium **without CsA** and plate  $20 \times 10^6$  cells/ flask in 40 mls 1° Splenic B cell Medium.

Day 9:

17. Plate  $7.1 \times 10^6$  irradiated CD-40 L cells in a T162 flask in 20 mls L cell medium.

Day 10:

18. Pour medium off L cells.

19. Collect splenocytes from each flask, count, centrifuge and decant.

20. Resuspend splenocytes in 1° Splenic B cell Medium **without CsA** and plate  $20 \times 10^6$  cells/ flask in 40 mls 1° Splenic B cell Medium.

Day 13:

21. Harvest B cells (can phenotype with anti-B220).