C

METHOD

ration of Cells from Adult X. laevis

Isolation of Peritoneal Leukocytes

An example of a scatter profile for peritoneal leucocytes is shown in Figure 1B.

1. G : E *X*
   A F L (G 2018).
2. 0.4% A B (H 7.3). A 10 L :
   10- L . L .
   Cells that have taken up trypan blue are nonviable.

Isolation of Blood Leukocytes

3. G 1–2 L A B , 50 / L . A
   f 4 L.
4. A 4 L 51% (/) 1.130 0.005 / L A B 15- L
   . C 4 L / /A B .
5. G , 300 25 4°C.
   Centrifugation over the Percoll gradient should be performed with slow acceleration and a slow or no break to prevent disruption of the interphase.

6. G [illegible] 1- L [illegible]
   1.5- L [illegible] (E, . 1A).
7. 300 5, 4°C. [illegible] 1, L A B . M [illegible] .
8. [illegible] 7 .
9. 2.

Isolation of Leukocytes from Lymphoid Tissue

An example of a scatter profile for spleen cells is shown in Figure 1C.

10. H [illegible] .

45 A B . G
1.5 L .

12. 300 5 4°C. D .
Resuspend the pellets in APBS and combine in a final volume of 1 mL.

13. 2.
To remove red blood cells from the sample, Percoll gradient separation (Steps 4–9) can be performed.

Isolation of Leukocytes from Nonlymphoid Tissue

14. H A B .
15. C 1–2 15- L 5 L A B .
16. A ( ), A B fi . D .
17. 300 5 4°C. D . A 4 L A B .
18. 4–9.

Staining Procedure

Prepare the following controls: (1) unstained cells; (2) cells incubated with an isotype control secondary antibody alone; (3) cells incubated with fluorochrome-conjugated streptavidin alone; and (4) fluorochromes minus one control, i.e., prepare samples that have a combination of all fluorochromes used in the experiment except one. Depending on the number of fluorochromes used, the number of fluorochromes minus one control will vary. For example, in a three color staining protocol with anti-CD8-FITC, anti-CD4-PE, and anti-CD3-APC, three fluorochromes minus one control are needed (1. anti-CD8-FITC and anti-CD4-PE; 2. anti-CD8-FITC and anti-CD3-APC; and 3. anti-CD4-PE and anti-CD3-APC).

A schematic showing different staining procedures (i.e., indirect and direct staining) is shown in Figure 2.

19. D FC B 1 106–1 107 / L. A 100 L FAC .
20. A 100 L 50–100 / L . M .
21. I 30 .
22. 300 5 4°C. D .
Resuspend the cells gently in 1 mL of FCSB.
23. 22 100 L FC B.
24. D fl 1:100 (0.1–1 / L) 100 L FC B. A I 30 .
Titrate the secondary antibody to determine which dilution allows for the strongest specific signal with the least background.
25. 300 5 4°C. D . I 1 L FC B.
26. I 25 100 L FC B.
If single staining is performed, proceed to Step 36.
27. B M (2010) 1 / L FC B. A 100 L .

Perform a titration curve to determine which dilution of the biotinylated primary antibody allows for the strongest specific signal with the least background.

28. I [illegible] 30 [illegible].
29. [illegible] 300 × 5 [illegible] 4°C. D [illegible].

Resuspend the cells gently in 1 mL of FCSB.

30. I [illegible] 29 [illegible] 100 µL FC B.
31. D [illegible] 1/100 FC B 100 µL [illegible].
32. I [illegible] 30 [illegible].
33. [illegible] 300 × 5 [illegible] 4°C. D [illegible].

Resuspend the cells gently in 1 mL of FCSB.

34. I [illegible] 33 [illegible] 400 µL FC B.
35. J [illegible], 0.5 µL [illegible].
36. A [illegible] FAC [illegible].

Problem (Step 36): B [illegible] fi [illegible].
Solution: I [illegible] B A [illegible] FC B [illegible] 2%–5%. A [illegible] 0.5% [illegible] 30 [illegible].

DISCUSSION

[illegible]

Flow Cytometric Analysis of *Xenopus* Immune Cells

Eva-Stina Edholm

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