

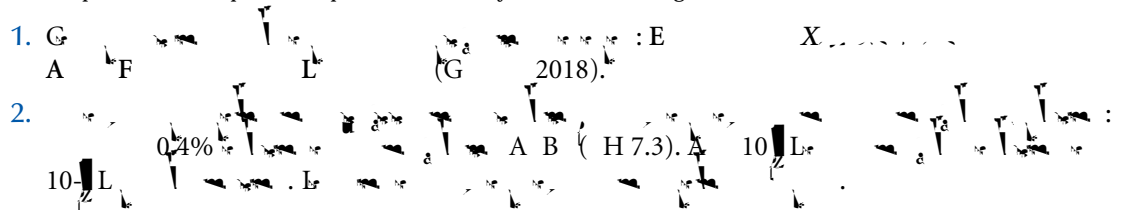


METHOD

Isolation of Cells from Adult *X. laevis*

Isolation of Peritoneal Leukocytes

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



Cells that have taken up trypan blue are nonviable.

Isolation of Blood Leukocytes

1. G 1-2, L_e 4, A B 50 / L_e 15, A 1
2. A 4, L_e 51% (/), 1.130, 0.005, / L_e 15, L_e 4, L_e / / A B
3. 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
 1.5 L (1A).
 7. 300 5 4°C.
 8. 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

12. Resuspend the pellets in APBS and combine in a final volume of 1 mL.
13. 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. D 1–2, 15; L A B .
16. A (), A B .
17. 300 5, 4°C. D . A 4, L
18. 4–9.

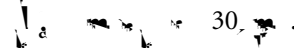
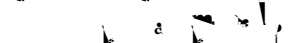

Staining Procedure

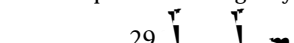
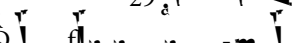
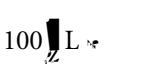

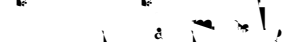

Prepare the following controls: (1) unstained cells; (2) cells incubated with an isotype control secondary antibody alone; (3) cells incubated with fluorochrome-conjugated streptavidin alone; and (4) fluorochromes minus one control, i.e., prepare samples that have a combination of all fluorochromes used 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).

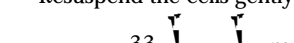
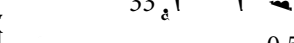

A schematic showing different staining procedures (i.e., indirect and direct staining) is shown in Figure 2.

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

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

- 28.  30.
- 29.  300 5 4°C. 

Resuspend the cells gently in 1 mL of FCSB.
- 30.  100 FC B.
- 31.  1/100 FC B.  100
- 32.  30.
- 33.  300 5 4°C.  100

Resuspend the cells gently in 1 mL of FCSB.
- 34.  400 FC B.
- 35.  0.5
- 36.  FAC

Problem (Step 36): B

Solution:

30. B A FC B 2%–5%. A 0.5%

DISCUSSION



Flow Cytometric Analysis of *Xenopus* Immune Cells

Eva-Stina Edholm

Cold Spring Harb Protoc; doi: 10.1101/pdb.prot097600 originally published online April 18, 2018

Email Alerting Service

Receive free email alerts when new articles cite this article - [click here](#).

Subject Categories

Browse articles on similar topics from *Cold Spring Harbor Protocols*.

- [Antibodies](#) (56 articles)
- [Cell Biology, general](#) (1323 articles)
- [Flow Cytometry](#) (40 articles)
- [Immunology, general](#) (112 articles)
- [Immunostaining](#) (113 articles)
- [Immunostaining Cells](#) (38 articles)
- [Immunostaining, general](#) (75 articles)
- [Xenopus](#) (146 articles)

To subscribe to *Cold Spring Harbor Protocols* go to:
<http://cshprotocols.cshlp.org/subscriptions>