5-bromo-2-deoxyuridine (BrdU) and 7-amino-actinomycin (7-AAD) staining for cell proliferation assay

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Introduction

The incorporation of 5-bromo-2-deoxyuridine (BrdU) in replicating DNA of proliferating cells can be used to measure their proliferation. The following protocol allows the identification of cells which have incorporated BrdU by flow cytometry.

Reagents

BrdU (Roche)

Frosted microscope slides (Fisher Scientific)

RPMI-1640 medium (Cellgro)

ACK Lysing Buffer (Cambrex)

Cell strainers, 70 µm nylon (Becton Dickinson)

CD43 (Ly-48) magnetic beads and separation columns (Miltenyi)

0.5% Paraformaldehyde (Sigma) in PBS

3 N HCl/0.5% Tween 20 (freshly made, remember that concentrated HCl is 12 N)

0.1 M Disodium tetraborate (Sigma)

FACS buffer: PBS + 0.5% fetal calf serum (Sigma) + 5mM EDTA (Sigma)

FITC-labeled anti-BrdU (Pharmingen)

7-AAD solution (Pharmingen)

Procedure

Injection of BrdU into mice

1 Inject 1 mg of BrdU, dissolved in 300 µl PBS, into the peritoneal cavity 12 h before analysis. Eventually, it is useful to start with injections 24 h before analysis and to inject a second time 12 h later.

Preparation and analysis of cells

▲Perform the following steps at 4 ˚C unless indicated otherwise.

2 Harvest splenocytes by grinding of spleens in RPMI-1640 medium between two frosted microscope slides. Spin cells at 1000 RPM for 5 min. Remove erythrocytes by adding one equivalent volume of ACK Lysing Buffer to the remaining pellet. Gently resuspend, swirl tube for 1 min. Fill tube with 10 ml of RPMI-1640, centrifuge at 1000 RPM for 5 min. Resuspend in 10 ml of RPMI-1640. Filter through 70

μm nylon cell strainer. Centrifuge at 1000 RPM for 5 min. Resuspend 1 x 10^7 cells in 500 μl of FACS buffer. Obtain B cells by CD43 negative purification using anti-CD43 magnetic beads.

3 Wash and resuspend 1×10^6 splenic B cells in 500 μl of 0.5% paraformaldehyde in PBS. Incubate on ice for 20 min (fixation). Spin and wash once with PBS.

4 Cell permeabilization: Spin and resuspend in 1 ml of freshly made 3 N HCl/0.5% Tween 20. Incubate at 25 ˚C for 20 min.

5 Spin and resuspend in 1 ml of 0.1 M disodium tetraborate. Spin and wash with FACS buffer.

6 Stain with FITC-labeled anti-BrdU. Incubate on ice for 20 min.

7 Spin and resuspend cells in 500 μl FACS buffer containing 2.0 μl of 7-AAD solution.

8 Analyze 10 min later.

▲NOTE: Because there is no dehydration step in this protocol, you still need to gate on the proper populations on forward scatter versus side scatter.

Timing

For preparation and analysis of cells, 2 h

References

1. Anzelon, A. N., Wu, H. & Rickert, R. C. Pten inactivation alters peripheral B lymphocyte fate and reconstitutes CD19 function. *Nat Immunol* **4**, 287-294 (2003).
2. Sanchez, Y. *et al.* Humoral and cellular immunity to hepatitis B virus-derived antigens: comparative activity of Freund complete adjuvant alum, and liposomes. *Infect Immun* **30**, 728-733 (1980).
3. Engel, P. *et al.* Abnormal B lymphocyte development, activation, and differentiation in mice that lack or overexpress the CD19 signal transduction molecule. *Immunity* **3**, 39-50 (1995).
4. Gallagher, E. D., Xu, S., Moomaw, C., Slaughter, C. A. & Cobb, M. H. Binding of JNK/SAPK to MEKK1 is regulated by phosphorylation. *J Biol Chem* **277**, 45785-45792 (2002).

5. Gallagher, E. D., Gutowski, S., Sternweis, P. C. & Cobb, M. H. RhoA binds to the amino terminus of MEKK1 and regulates its kinase activity. *J Biol Chem* **279**, 1872-1877 (2004).

6. Rush, J. S., Hasbold, J. & Hodgkin, P. D. Cross-linking surface Ig delays CD40 ligand- and IL-4-induced B cell Ig class switching and reveals evidence for independent regulation of B cell proliferation and differentiation. *J Immunol* **168**, 2676-2682 (2002).

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Kinase MEKK1 is required for CD40-dependent activation of the kinases Jnk and p38, germinal center formation, B cell proliferation and antibody production

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