

## BrdU Staining

protocol by Susannah D. Barbee

Label thymocytes by continuous administration of BrdU (Sigma B5002) via drinking water at 0.8 mg/mL for the desired number of days<sup>†</sup>.

1. Surface stain with PE- and CyChrome-conjugated antibodies (use 1x PBS as buffer), wash 1x PBS (Cf 6-10 min 1200 rpm).
2. Resuspend in 500  $\mu$ L 0.15 M NaCl (ice cold), keep on ice.
3. Add 1.2 mL 95% EtOH (-20°C) **dropwise** while vortexing.
4. On ice 30 min.
5. Wash 1x 2 mL PBS.
6. Resuspend in 1 mL PBS/1% paraformaldehyde/0.01% Tween-20.
7. ON 4°C.
8. Cf (increase to 2000 rpm).
9. Resuspend in 1 mL DNase:

0.15 M NaCl	1:10	1.5 M
4.2 mM MgCl <sub>2</sub>	1:10	42 mM
10 $\mu$ M HCl	1:1000	10 mM
50 Kunitz units/mL DNase I	1:100	frozen stock
10. 30 min 37°C.
11. Top off with PBS (RT), Cf.
12. Resuspend in 20  $\mu$ L  $\alpha$ BrdU FITC or isotype control (Pharmingen 36634K) .
13. 30 min RT.
14. Wash 1x PBS, resuspend in PBS.

<sup>†</sup> BrdU should be stored as a powder in the dark at -20°C in a desiccator. Before an experiment, I usually weigh out 40  $\mu$ g of BrdU per tube for several 50 mL conical tubes, which are sealed with parafilm and stored in the dark at -20°C. Each day, I suspend the BrdU in 50 mL of H<sub>2</sub>O by a brief incubation at 37°C. Rubber stopper tops of mouse sipper bottles will fit the conical tubes. The BrdU-labeled drinking water should be changed frequently due to the exposure to light (preferably every day).