

# BrdU and Cell Surface Labeling Protocol

## Mouse Thymocytes

### Reagents

HBSS	1% Tween 20
150mM NaCl	Rat anti-Mouse CD4-PE
HBSS+ (HBSS + 1% BSA)	Rat anti-Mouse CD8-PE/Cy5
anti-BromodeoxyUridine mAb-FITC ( $\alpha$ -BrdU-FITC)	10mg/ml Bromodeoxyuridine
DNase buffer: 4.2mM MgCl <sub>2</sub> in 150mM NaCl, pH8.5	2% PFA (fresh paraformaldehyde)
<b>FIX:</b> 1.0% PFA + 0.1% Tween 20 in HBSS	100% Ethanol
	DNase

### Procedure

1. Inject mouse IP twice with BrdU with a four hour interval between injections. Allow 30' after last injection before sacrifice. (100ul of 10mg/ml BrdU/HBSS)
2. Prepare a single cell suspension and cell surface label CD4/8 by standard protocol.
3. Wash cells 1 in HBSS+ and 2X in HBSS.
4. Re-suspend cells in 400ul of 150mM NaCl and 850ul 100% Ethanol. Incubate 30' on ice.
5. Wash 1X in 2mls HBSS.
6. Re-suspend cells in **FIX** for 3 days at 4<sup>0</sup>C.  
(**FIX:** 200ul HBSS & 250ul 2.0% PFA & 50ul 1% Tween 20).
7. Wash cells 2X in HBSS. Incubate cells in DNase & DNase buffer (57U/tube in 100ul buffer) at 37<sup>0</sup>C for 40'.
8. Wash cells 2X in HBSS and 2X in HBSS+.
9. Intracellular labeling with  $\alpha$ -BrdU-FITC. 1:25 dilution in HBSS+ with a final volume of 50ul/tube. Incubate 60' on ice.
10. Wash 2X with HBSS+ and 2X with HBSS. Re-suspend cells in a final concentration of 1% PFA. Provide the proper controls and analyze by flow cytometry.