

# Cell Surface Immunophenotyping & DNA Content Protocol

## Reagents

2% NCS/0.1% NaAz (sodium Azide)/PBS	<b>7-AAD Master Mix</b>
PBS, Ca <sup>++</sup> , Mg <sup>++</sup> -free	PBS 975 ul
0.2% Tween 20/PBS	<b>7-AAD</b> 25 ul
Rat anti-Mouse CD4-PE	1,000 ul
Rat anti-Mouse CD8-FITC	
7-Amino-Actinomycin D ( <b>7-AAD</b> ) 1mg/ml stock:	[ <b>7-AAD</b> ] <sub>F</sub> = 25ug/ml
2% Formaldehyde(aka PFA)/PBS heated to clarify and 0.45um filtered).	

## Procedure

1. Prepare a single cell suspension and Cell Surface Label  $1 \times 10^6$  cells (FITC/PE).
2. Wash cells 2X in cold PBS and pellet by centrifugation ( $\sim 1,200$  rpm,  $5^\circ$ ,  $4^\circ\text{C}$ ).
3. Fix surface immunofluorescence by re-suspending cells in 0.875mls cold PBS and add 0.125mls cold, fresh 2% PFA while vortexing. Incubate  $60'$ ,  $4^\circ\text{C}$ .
4. Pellet cells and permeabilize cytoplasmic and nuclear membranes by re-suspending cells in 1ml 0.2% Tween 20/PBS at room temp. Incubate cells  $60'$ ,  $4^\circ\text{C}$ . Pellet cells.
5. Re-suspend cells in **7-AAD** at a final cell density of  $0.5 \times 10^6$  cells/ml. Transfer to a Falcon 2054 (Fisher # 149592A) tube, incubate  $30'$  at room temp. And analyze within 2 hours.
6. Supply a wash tube of **7-AAD** at 25ug/ml. Provide approx. 1ml **7-AAD** soln. for every 5 samples.

## References

Schmid, I., Uittenbogaart, C.H., and Giorgi, J.V. (1991) **A Gentle Fixation and Permeabilization Method for Combined Cell Surface and Intracellular Staining With Improved Precision in DNA Quantification** *Cytometry* 12: pp. 279-285

Rabinovitch, P.S., Torres, R.M., and Engel, D. (1986) **Simultaneous Cell Cycle Analysis and Two-Color Surface Immunofluorescence Using 7-Amino-Actinomycin D and Single Laser Excitation: Applications to Study of Cell Activation and the Cell Cycle of Murine Ly-1 B Cells.** *J. Immunol.* 136:8 pp.2769-2775