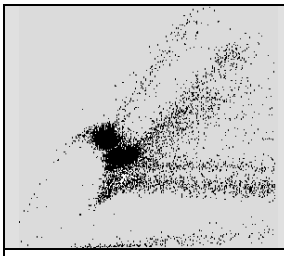

THE GOODELL LABORATORY

Author	Olga Sirin	Month Day, 1996
Title	**Pyronin Y and Hoechst 33342 Staining of BM cells using fixation**	
Introduction	This protocol describes the use of the Pyronin Y and Hoechst 33342 for cell cycle analysis with separation of Go and G1. Pyronin Y binds RNA and DNA, however in the presence of a dye that binds DNA, in this case Hoechst, it binds only the RNA. This allows for separation of the populations in Go and G1, unlike with regular propidium iodide staining that shows a peak containing both populations.	
Materials	1. HBSS+ : Hanks Balanced Salt Solution (from Gibco) with 2% Fetal Calf Serum and 10 mM 2. Pyronin Y 3. Hoechst 33342: …….. 4. ……..	
Protocol		*Notes*
1.	Isolate BM cells (one mouse)	
2.	Enrich for Sca-1 using microbeads. (If you do not want to Sca enrich jump to step 8). Stain with Sca-1 biotin Ab for 15 min on ice.	
3.	Wash with cold HBSS+	
4.	Resuspend pellet in 800µl HBSS+ and 200µl microbeads and store in 4°C for 15 min.	
5.	Wash cells with HBSS+ and resuspend in 0.5ml. Run “posselds” on automacs.	
6.	Wash enriched cell population with cold HBSS+	
7.	Resuspend in 1 ml HBSS+ and stain with desired cell surface markers on ice for 15 min. Then wash with HBSS+ as before.	*I stain with ckit-APC since it is only one of the few compatible colors ie Pyronin Y bleeds into PE-cy5 so do not use Abs conjugated to that.*
8.	Agitate pellet.	

9.	Vortex pellet as you drop wise add ~5ml 70% -20°C ETOH. Place cells in -20°C 1hr-ON	*This is an important step, as you do not want any clumping of cells*
10.	Wash cells in Hanks+	
11.	Resuspend in Hanks+ with 2µg/ml Hoechst and 4µg/ml Leave cells in dye for a minimum of 1hr.	
12.	Ready for flow	