

Protocol for staining cells with Hoechst 33342 and Pyronin Y

This protocol is commonly used in stem cells (although can be used in any other cell type) to differentiate G1 from G0 cells in the cell cycle, and stains both DNA (Hoechst) and RNA (Pyronin).

Note: This requires a UV excitation source

1. With cells in culture, aspirate media if they are adherent. If floater cells, spin down and resuspend in new media.
2. [If cells are from an animal, then remove from animal, wash 1x with PBS, lyse red cells w/ ACK lysis buffer, wash 1x w/ PBS, and proceed, with cells at approximately 1-2 million per mL in media (for mice, IMDM or RPMI w/ 5-10% FCS works well). You MUST lyse before Hoechst addition. Lysing after you add Hoechst will ruin your experiment.]
3. Replace with fresh, warm media.
4. Add Hoechst 33342 dye – to a final concentration of 10 ug/mL (stock comes as 10mg/ml, which is 10ug/uL, so adding 1ul for every 1mL of media is the easiest way).
5. Incubate at 37 degrees Celcius for 45 minutes.
6. Either remove media and trypsinize then wash for adherent cells, or spin down and wash 1x w/ media for floater cells.
7. If staining for extracellular markers (i.e.: Sca-1, Lineage, c-kit, etc) then resuspend cells in 300mL of PBS, and stain cells on ice in dark for 30 minutes with antibodies. (N.B.: You can not use a PE antibody, as Pyronin Y fluorescence is in this channel, and you can not use a UV antibody, as Hoechst is in this channel.)
8. After staining, or if just spun down w/o staining, resuspend in 2mL of 5% paraformaldehyde.
9. Samples must sit at least overnight in 5% PFA in fridge, can sit for 1-2 weeks fine too.
10. When you want to add PY (best PY is from Polysciences, Inc. – it is what the Scadden lab uses, and is what this protocol and dilutions are written for), make 1:100 dilution then add 10uL to each mL PFA (so, final dilution is 1:1000 from stock – but for some reason, it looks better if dilution is done in this stepwise fashion rather than dye added directly to cells from stock!)
11. Put tube in fridge for 30 minutes to incubate w/ PY.
12. Spin down, resuspend cells in 500mL 5% PFA, analyze.
13. When analyzing, remember that antibody fluorescence is log scale, PY fluorescence and Hoechst fluorescence are linear scale.