

Staining for Cell Cycle using Hoechst 33342

Uses and Rationale:

Like other DNA dyes, Hoechst 33342 binds to the DNA and allows you assess cell cycle. Hoechst crosses the cell membrane of viable (and non-viable) cells and binds DNA allowing one to assess cell cycle. Because it traverses live cell membranes, it is not good for viability protocols. Used with Pyronin Y (which stains RNA), Hoechst dyes can be used to discriminate between G0/G1 cells.

Materials:

Hoechst 33342
Potentially: Verapamil
37 degree water bath/incubator
Warm RPMI/10% FCS

Method:

1. With cells in culture, aspirate media if they are adherent. If floater cells, spin down and resuspend in new media.
2. Replace with fresh, warm media.
3. Add Hoechst 33342 dye – to a final concentration of 10 ug/mL.
4. Incubate at 37 degrees Celcius for 45 minutes (if your cells overexpress Pgp, a Pgp inhibitor should be added to the media. Ask if you are unsure).
5. Either remove media and trypsinize then wash for adherent cells, or spin down and wash 1x w/ media for floater cells.
6. Stain for extracellular markers if appropriate, on nice, in dark.
7. Wash and run, or fix.

Stock: 10mg/ml

Add: 1ul for every 1mL of media, 1-2x10⁶ cells/ml

Caution: No known for this compound

Important technical notes:

This protocol is wonderful for staining cells transfected/transduced with GFP, YFP, RFP, etc. because you do not have to permeabilize the cell. As such, the fluorescent protein does not leak out of the cell, and you can preserve the fluorescence of the native protein as well as get a DNA profile.

This requires a UV excitation source (325-350nm laser line), and as such, has to be arranged in advance w/ a sorter operator or done on a cytometer with a UV light source (LSR or Cyan). This dye is added to cells in media or in culture (at 37 degrees usually) then washed out. Some have fixed cells after this and run them the next day, and they look great, but longer than that and they have looked poor (wide CVs).