SYTO9 & PI FACS Viability Assay

SYTO9/PI Viability Protocol

- stock solutions of SYTO9 (3.34 mM) and Propidium Iodide (PI, 20 mM) stored at -20°C in the Gresham Lab

1. collect desired cell samples by spinning @10,000g for 5mins and resuspended in water
2. sonicate samples and measure cell density by coulter counter
3. resuspend cells in 1XPBS with desire volume to achieve the cell concentration $10^6$ – $10^7$ cells/ml
4. prepare two 1 ml sample with $10^6$ – $10^7$ cells, one for staining and one for blank control
5. allow the dye to thaw at room temperature in the dark
6. add 1ul of STYO9 and PI in 1ml staining sample (final conc. SYTO9: 3.34uM; PI: 20uM) and vortex gently
7. incubate samples at room temperature for 20mins in the dark
8. run sample through Flow cytometry or FACS

*note about use of blanks: one blank can be collected for each condition that will be measured (i.e. different strains, different media) OR can collect a blank for each SAMPLE (i.e. each time point) being measured.

*can store samples in media and dye at 4°C protected from light for approximately 7 days until FACS analysis (may affect signals)

Testing the assay:

if desired, the assay can be tested for the conditions under which viability is being measured by doing a heat killed calibration experiment:

- grow up cell samples in the desired condition and create 1 mL samples of mixtures of 0%, 25%, 50%, 75%, and 100% viable cells
- 0% sample consists of cells that are completely non-viable (heat killed in 70-80°C water bath for 20-30 min.)
- 25% viable sample consists of 25% live cells from your culture and 75% heat killed cells
- 100% sample consists of all viable cells from your culture, etc.