Amine/sulfhydrl staining (protein content proxy) with FITC

This protocol is an adaptation of Porro et al 2003 and Cipollina et al 2005, and has been re-optimized for our lab/cytometer.

All spins are at 6000 rcf 1min RT. You will need 0.5M Na bicarbonate solution, 1x PBS (preferably filtered), FITC stock solution (5mg/ml in DMSO).

KEEP ALL FITC UNDER FOIL AS MUCH AS POSSIBLE. This protocol is a little time sensitive. Fluorescin (FITC) will degrade in water, so be consistent with your timing between samples especially after adding FITC to the bicarbonate buffer. Do consider running controls of FY4 in YPD exponential, in order to normalize between different days.

- Perform an Ethanol fix in whatever condition you want. The following assumes that cells are be in 70% etOH and at -20C. You of course need to count the density of these fixed cells to proceed.
- Put 5 million (5*10^6) cells in an eppendorf tube. Spin them down. If they're not pelleted, spin longer. If they're not spun down by 10min, check if you actually have cells.
- Aspirate all of the fixative, R/S in 1x PBS 1ml. Spin down.
- Aspirate. R/S in 500ul 0.5M Na bicarbonate. Add 5ul of the 5mg/ml FITC solution (~ 1 in 100 dilution). Let sit RT 30min (UNDER FOIL).
- Spin down.
- Aspirate ALL OF THE STAINING SOLUTION, use a small tip. R/S in 1ml 1x PBS. Spin down.
- Aspirate. R/S in 1ml 1x PBS. Spin down.
- Aspirate. R/S in 0.5ml 1x PBS. Put on ice. You can adjust cell concentration (volume PBS) as needed for your flow cytometry.
- Sonicate all samples. You can use with the standard 5s protocol (power 10) and it works okay.
- Flow on the Accuri C6:

Make sure to bring unstained controls. The initial etOH-fixed cells R/S in 1x PBS works fine, looks like ones incubated in bicarbonate buffer. Consider a control with proteinase K treatment, if you want another control.

Here's an optimization run, done with freshly made FITC stock. See Darach's notebook #6 page 35. Used cells from a chemostat at 0.12 growth rate. Tried 50ug/ul, 10ug/ul, 5ug/ul, 1ug/ul, and 500ng/ul. The 10ug/ul looks like a fine concentration, but heavily saturating the reaction with 50ug/ul doesn't seem to negatively affect the reaction. I would recommend saturating, especially in case you make a mistake with the cell count and have some abnormally high protein content.

Here's the same cells at different staining concentrations (in ng/ml)

Here's the effect of proteinase K digest for 0, 20, 30min when stained at 5ug/ml
exp158fitoptimize151211.html