Metabolic activity assay -- FUN-1 staining

- stock solutions of FUN-1 (10mM) 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 GH solution 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 FUN-1 in 1ml staining sample (final conc. 10uM) and vortex gently
7. incubate samples at 37°C for 30mins in the dark
8. run sample through Flow cytometry or FACS

Glucose HEPES (GH) stock recipe (50ml stock):
- 2.5ml 40% Glucose -2% D-(-)-glucose (final conc.)
- 0.5ml 1M HEPES (pH7.2) -10mM sodium HEPES (final conc.)
- 47ml sterile ddH$_2$O

*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.

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.