Metabolic activity assay -- FUN-1 staining

- stock solutions of FUN-1 (10mM) stored at -20°C in the Gresham Lab
- collect desired cell samples by spinning @10,000g for 5mins and resuspended in water
- sonicate samples and measure cell density by coulter counter
- resuspend cells in GH solution with desire volume to achieve the cell concentration 10^5 – 10^7 cells/ml
- prepare two 1 ml sample with 10^5 – 10^7 cells. one for staining and one for blank control
- allow the dye to thaw at room temperature in the dark
- add 1ul of FUN-1 in 1ml staining sample (final conc. 10uM) and vortex gently
- incubate samples at 37°C for 30mins in the dark
- 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 ddH₂O

*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.