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Direct Bacterial Counts via Live/Dead Stain

Adapted from Kristine Johnson (Firestone lab) and Dominique Joyner (Hazen lab)

Conduct direct counts of active and total bacteria biomass using the two-part stain LIVE/DEAD.
SYTO 9 stains live bacteria (with intact membranes) green and propidium iodide stains dead bacteria red.

Supplies:

- LIVE/DEAD BacLight Bacterial Viability Kit (kit contains dyes and mounting oil)
  Molecular Probes cat # L-7007 go to : www.probes.com for refs, info etc.
- Black Polycarbonate Membranes 25mm 0.2um (available through VWR)
  Whatman Nucleopore cat # 110656
- Epifluorescence microscope
- **GFP/FITC** filter (ex:480nm em:500nm) and **rhodamine** filter (ex:490nm em:635nm)
- 25mm Filter Holder w fritted glass support (VWR # 26316-690)
- 15ml glass funnel (VWR # 26316-694)
- Aluminum clamp (VWR # 26316-700)
- Silicone stopper #5 (VWR # 26316-702)
- Filtering Flask (VWR # 26316-736).
- 10 ml PBS per sample

Stain: Prepare a 200X dilution of each dye (Component A and Component B) in filter-sterilized water (ie> 10ul A + 10ul B + 1980ul f.s. H2O); store solution for up to 2 weeks at 4C in the dark

Buffer: 50mM filer sterilized PBS

Methods:

1. Dilutions of soil

Make 10^{-3} -10^{-5} serial dilutions in 5mM bicarb buffer, sonicate for 15 minutes (1g in 10ml is 10^{0} dilution)

* With a new sample it is best to prepare 3 dilutions, your target dilution and one above and one below

*In selecting your optimal dilution, you want a dilution that will provide you with approximately 20-30 cells/field of view. In addition, you want to dilute the sample enough so that there is no overlap of soil particles in a field of view, which might cover cells.

*Be sure that sample is well mixed and that serial dilutions are accurate.

2. Stain - within 48 hrs of harvest.

Transfer 500ul of the final dilution to a sterile tube

Add 120ul of stain; incubate in the dark for 15 min.

After incubation, wash off all unbound dye by adding 10-15ml of your buffer to the tube, vortex gently and filter onto 0.2 µm pore size Black Polycarbonate membrane (using filtration apparatus)

3. Microscopy - within 24-36 hrs *of staining*

Slides were viewed at 1000X using epifluorescence microscopy

Mount membrane onto coverslip and view with epifluorescence microscope at 1000X

** I like to place a drop of oil onto the slide and put the coverslip over the drop (perpendicular to the slide) to spread the oil out flat… when you are ready to place your membrane on the slide, remove the coverslip by sliding it off to the side…. This way you have a nice thin film of oil that will serve to hold the membrane flat on the slide. Place a drop of oil on the membrane and use the same coverslip to cover the membrane

Use whichever dilution yields the highest biomass counts (in most cases, the 10^{-4} dilution).

View your samples quickly as photobleaching occurs. Keep them out of the light if there is a delay between preparation and counting. GOOD LUCK !!