

Laboratory 1: Bacterial Abundance Continued

Method 3: STAINING CELLS FOR DIRECT EPIFLUORESCENCE COUNTING

SOLUTIONS

4', 6 diamidino-2-phenylindole (DAPI)

Stock solution: 1000 $\mu\text{g/ml}$ (dissolve 5 mg in 5 ml 0.2 μm filtered DI water).

Working Solution: 200 $\mu\text{g/ml}$ (add 1 ml stock solution to 4 ml DI).

Filter the working solution through a 0.2 μm acrodisc

A filtered working solution has been prepared for you

Phosphate buffered saline (PBS)

METHOD

1. Using sterile forceps, place a glass fiber (Whatman GF/F) backing filter on the Millipore frit, and dampen with a drop of DI water (this filter can be reused). Place on top of the backing filter a 0.22 μm pore size black polycarbonate filter. Clamp on the 15 ml filter tower.
2. Mix 1.8 ml PBS and 0.2 ml of preserved sample in a sterile snap cap tube. In another snap cap tube place 2 ml of PBS. This will be your blank. Label accordingly.
3. Stain your cells and PBS blank for 5 minutes with 100 μl DAPI working solution. This is located in foil wrapped microcentrifuge tubes in the refrigerator.
4. After 5 minutes pour the sample into the Millipore tower. Pull the water through the filter using the hand pump, but **DO NOT ALLOW THE FILTER TO GO DRY PRIOR TO ADDING THE PBS RINSE BELOW**. Always use low pressure (<12 cm Hg) filtration to avoid breaking cells.
5. Rinse tube and sides of the filter tower with approximately 1 ml PBS. Allow all water to pass through filter.
6. Put a small drop of immersion oil on a microscope slide.
7. Using sterile forceps remove the filter from the frit and put it on top of the oil. **KEEP FILTER FACE UP**. Dot another drop of oil on a glass cover slip and place it on top of the filter, oil side down. Add a drop of oil to the top of the glass cover slip.
8. Label the slide with your initials, and the column number (or blank if it is one).
9. Count at least 5 fields in the ocular grid using the epifluorescence microscope using the 100x oil-immersion objective. If there are only a few bacteria per field then you should prepare another filter, but use a larger sample volume (1-2 ml, without PBS), and add DAPI proportionately (50 $\mu\text{l/ml}$). Likewise, reduce sample volume if there are too many bacteria to count.
10. Calculate the average number of cells per field (note the area of the gridded field is 100 μm by 100 μm), the working area of the filter (measure the inside diameter of the 15 mL tower), and the number of cells per milliliter.