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We use this protocol and it's working

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Evans blue assay to stain dead cells.

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ABSTRACT

Evans blue (Sigma, USA, catalog no: E2129) is a dye that can enter the compromised cell membranes of dead cells and stain them blue. I used this assay to differentiate dead cells in algal cultures of *Chlorella* and *Chlamydomonas* (the same protocol can be used for other green algae).

Evans blue assay to stain dead cells.

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Introduction

2 Evans blue (Sigma, USA, catalog no: E2129) is a dye that can enter the compromised cell membranes of dead cells and stain them blue. I used this assay to differentiate dead cells in algal cultures of *Chlorella* and *Chlamydomonas* (the same protocol can be used for other green algae).

Preparation

3 Prepare 1% Evans blue (w/v in MilliQ water) by dissolving 0.1 gm Evans blue in 10 milliliter MilliQ water (I use Falcon tubes). Filter the dye through 0.2 Micron filters using a syringe and filter assembly (bigger pore size filters can also be used). I filter the dye to remove large undissolved particles of dye. The solution (cover it with aluminium foil) is stable at room temperate for several months.

Assay

4 Take 900 µl algal culture in 1.5 milliliter Eppendorf tube (I avoid 2 milliliter Eppendorf tubes as in these tubes the cells do not pellet properly; see below). If the algal cultures are less dense (less than a million cells/ milliliter), then a few milliliter of culture can be centrifuged, and the cell pellet combined and suspended in 900 µl culture media.

5 Add 100 ul Evans blue (from 1% dye suspension prepared above).

6 Incubate the mixture at room temperature for 20 minutes, preferably in the dark.

7 Rinse the cells with MilliQ water to remove excess dye. First, centrifuge the stained cells at 7000 RPM for 2 minutes. Remove about 900 µl dye with a 1 milliliter pipette (do not disturb the cell pellet). Since the dye is blue, the cell pellet cannot be seen during the first wash. Add 1 milliliter MilliQ water and centrifuge the cells. The cell pellet should be visible now as the leftover dye is no more concentrated. Repeat the above steps 2 to 3 times (remove 900 to 950 µl dye from the supernatant using a pipette, add MilliQ water, and centrifuge). Leave the cell pellet (after final wash) in a small volume (10 to 20 µl). Take a small amount of cell pellet (ca. 5 µl) and mount it on a clean glass slide. Put a coverslip, press gently so that cells are dispersed and observe the cells using a microscope. I use an oil immersion lens (100 X; Axioskop 40, Carl Zeiss, Germany). Count blue (dead) cells and estimate the dead cells (%) from the total cells.