

Cell Counting Protocol

Syto-9 is a green fluorescent dye that binds nucleic acid and is cell permeant. All nucleated cells will be positive for Syto-9. Propidium Iodide is a red fluorescent dye that binds nucleic acids and is cell impermeant. Only dead cells (or cells with a compromised cell membrane) will be stained red.

- Take 200ul of water sample and place into a 1.5mL eppendorf tube □
- Add Syto-9 dye (final concentration - 3.3 micomolar) to sample □
- Add Propidium Iodide (PI) (final concentration 20 micromolar) to sample
- Incubate 15minutes at room temperature □
- Analyze samples on ImageStreamX MkII using the 488nm laser line □ ImageStreamX MkII settings: Collect data using the Green and Red filters and 488nm excitation using the 60X Objective. Analyze data for shape (bright field area), fluorescence intensity (green and red intensity levels) and number of cells (objects/mL calculated using the volumetric syringe pump). Convert the objects/mL into objects/uL

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