Live/Dead Analysis of *Drosophila* Kc167 cells using Live/Dead Fixable Violet Dye on the Attune Cytometer

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Drosophila Kc167 cells (https://dgrc.cgb.indiana.edu/product/View?product=1) were cultured for 3 days in complete medium at 25oC. (Schneider's Medium Gibco #21720-024, 10% heat-inactivated FBS HyClone SH30070.03, 1X Pen-Strep Gibco #15070-063)

An aliquot of cells were fixed with ethanol (95%) at 4oC for 30 min.

The fixed cells (1 mL) and live cells (1 mL) were washed with 1mL of 1X Ca++, Mg++ free PBS pH7.2.

The fixed cells were mixed with live cells in different ratios (0:1, 1:1, 1:3, 3:1, 1:0) in 1 mL 1X PBS to a final concentration of 400,000 cells per ml.

One microliter of reconstituted Live/Dead Fixable Violet dye (L34955) was added to each 1 mL of cells.

Cells were incubated at room temperature for 20 min and 4oC overnight before analysis*.

*We find the overnight incubation at 4oC to be essential for dye incorporation into dead *Drosophila* Kc167 cells.

Data was verified using Trypan blue (T10282) staining and traditional cell counting with a hemocytometer.