

Live Cell Cycle Analysis of *Drosophila* Kc167 cells using Hoechst 33342 or
VybrantDyeCycle Violet on the Attune Cytometer

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Drosophila Kc167 cells (<https://dgrc.cgb.indiana.edu/product/View?product=1>) were cultured for 24 hours in complete medium at 25°C. (Schneider's Medium Gibco #21720-024, 10% heat-inactivated FBS HyClone SH30070.03, 1X Pen-Strep Gibco #15070-063)

Cells were plated at a density of 1×10^6 cells/mL in complete medium containing either DMSO (control) or 20 μ M SP600125 (Sigma S5567) to increase G1 population, for 24 hours. (Note that in contrast to mammalian cells, proliferating *Drosophila* cells are primarily in G2 phase¹).

Cells were re-suspended by gentle pipetting (*Drosophila* Kc167 cells are semi-adherent).

100 μ L of re-suspended cells were added to 1mL of Dulbecco's 1X PBS (Invitrogen 14190-144) containing either no dye, 10 μ L of 0.5 mg/mL Hoechst 33342 (Sigma B2261)* or 0.5 μ L of Vybrant DyeCycle Violet (Invitrogen V35003)**.

Cells were incubated at room temperature for 10 minutes.

Cells were run on the Attune cytometer using standard sensitivity at a flow rate of 100 μ L/min.

* We find that increasing the Hoechst concentration from the standard Live *Drosophila* cell protocol² improves Hoechst 33342 detection with the Violet Attune laser excitation.

** We find that a final concentration of 0.5 μ L/mL DyeCycle Violet provides sufficient DNA staining without the toxicity to *Drosophila* cells we observed at higher concentrations.

- 1 Echalier, G. *Drosophila: Cells in Culture*. (Academic Press, 1997).
- 2 de la Cruz, A. F. & Edgar, B. A. Flow cytometric analysis of *Drosophila* cells. *Methods Mol Biol* **420**, 373-389, doi:10.1007/978-1-59745-583-1_24 (2008).