Cell Cycle Analysis of *Drosophila* Kc167 cells using Alexa 488 Click-IT EdU and FxCycle Violet on the Attune Cytometer

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Drosophila Kc167 cells (https://dgrc.cgb.indiana.edu/product/View?product=1) are cultured in complete medium at 25oC. (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 $1X10^6$ cells/mL in a 6-well plate in complete medium containing either DMSO (control) or 20 µM SP600125 (Sigma S5567) to arrest cells, for 48 hours. (Note that in contrast to mammalian cells, proliferating *Drosophila* cells in complete medium are primarily in G2 phase[1]).

Cells were exposed to 10µM EdU at 25oC in complete medium for 30 min.
Cells were re-suspended by gentle pipetting and transferred to an eppendorf (Drosophila Kc167 cells are semi-adherent).

Staining Protocol:

- Pellet cells by centrifugation at 1,000K RPM room temp. for 5 min., and discard media.
- Wash cells with 1mL 1XPBX+1%BSA
- Pellet cells at 1000K RPM room temp. for 5 min. and discard the wash.
- Resuspend cells in 50µL of Click-IT fixative, incubate at room temp. for 15 min.
- Pellet cells at 1000K RPM room temp. for 5 min. and discard the fix.
- Resuspend pellet in 1mL 1XPBX+1%BSA to wash.
- Pellet cells at 1000K RPM room temp. for 5 min. and discard the wash.
- Resuspend pellet in a second wash of 1mL 1XPBX+1%BSA.
- Pellet cells again at 1000K RPM room temp. for 5 min. and discard the 2nd wash.
- Resuspend pellet in 30 µL of 1X Saponin Permeabilization buffer.
- Immediately prepare Click-IT EdU cocktail: (use 150 µL cocktail per sample, for 500 µL cocktail prepare <u>in order</u>):
- 438 µL 1XPBS
- 10 µL CuSO4
- 5 µL 488-Click-IT Azide (this is 2X the standard protocol, our lab stock is diluted 2X)
- 50 µL 1X Buffer additive (this must be freshly prepared from 10X stock by dilution with dH2O and used within 15 min. of dilution)

- Mix well, add 150 µL cocktail per sample. –Must be used within 15 min. of preparation.

- Incubate for 30 min. room temp. protected from light.
- Pellet cells at 1000K RPM room temp. for 5 min. and discard supernatant.
- Wash pellet in 100 µL of 1X Saponin Permeabilization buffer.
- Pellet cells again at 1000K RPM room temp. for 5 min. and discard wash.
- Resuspend pellet in 100 µL of 1X Saponin Permeabilization buffer.
- Dilute FxCycle Violet in 1:1,000 in 1XPBS. Add 1mL FxCycle Violet in 1XPBS to each sample in 100 µL of 1X Saponin Permeabilization buffer.
- Incubate at room temp. for 30 min.

- Run cells on the Attune cytometer using standard sensitivity at a flow rate of 100uL/min. (Cells can be run on cytometer directly from the eppendorf.)