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 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 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 25°C 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 in order):
  - 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.)