

Materials List for:

Live Cell Cycle Analysis of *Drosophila* Tissues using the Attune Acoustic Focusing Cytometer and Vybrant DyeCycle Violet DNA Stain

Kerry Flegel^{*1}, Dan Sun^{*1}, Olga Grushko^{*1}, Yiqin Ma¹, Laura Buttitta¹

¹Molecular, Cellular and Developmental Biology, University of Michigan

*These authors contributed equally

Correspondence to: Laura Buttitta at buttitta@umich.edu

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Materials

Name	Company	Catalog Number	Comments
12x75 mm Polystyrene Round-Bottom 5 ml Test Tube	BD Falcon	352058	5 ml tubes
Attune Acoustic Focusing Cytometer	Life Technologies/ Applied Biosystems	4445315	Blue / Violet configuration
Attune Cytometer Software (version 1.2.5)	Life Technologies/ Applied Biosystems	Free	PC only
Attune Performance Tracking Beads (5 x 106 beads/ ml)	Life Technologies/ Applied Biosystems	4449754	For daily performance test
Dumont #5 Inox forceps	Fine Science Tools	11251-20	
Embryo dishes 30 mm x 12mm	Electron Microscopy Sciences	70543-30	Glass dissection dishes
Eppendorf Thermomixer	Eppendorf	022670051	
Trypsin-EDTA Solution (10x)	Sigma	T4174	
Vannas-Tübingen Spring Scissors	Fine Science Tools	15003-08	Straight 5mm Cutting Edge
Vybrant DyeCycle Violet Stain	Life Technologies/ Invitrogen	V35003	

Table 1. Required reagents and instruments.

Live DNA Stain Solution (10 ml):

1 ml 10X Ca²⁺ Mg²⁺ free PBS (pH7.2)
 9 ml 10X Trypsin-EDTA (Sigma)
 5 µl Invitrogen Vybrant DyeCycle Violet (note that this is 0.25X the recommended concentration for mammalian cells. We find that higher concentrations are toxic to *Drosophila* cells.)

10X Ca²⁺ Mg²⁺ free PBS (pH7.2): 1.37M NaCl, 27 mM KCl, 100mM Na₂HPO₄ (dibasic), 20mM KH₂PO₄ (monobasic) adjusted to pH 7.2

	FSC	SSC	BL1	VL1
Threshold (x1000)	100	10	10	10
Voltage (mV)	2950	4250	1800	1150

Table 2. Threshold and voltage setting for the analysis in Figure 2.