**PI Stain for mammalian cells (Cell Cycle Analysis)**

**Nim Buffer (50ml)**

BSA - 0.5% w/v (.25g)

NP-40 – 0.2% w/v (10ul of 10% stock)

1xPBS pH 7.4 (5ml)

**Add fresh to buffer (13ml):**

 RNAse AT/1 – 200 ug/ml (260 ul of 10 mg/ml stock)

 PI – 25ug/ml (325 ul of 1 mg/ml stock)

* Remove media by pipet and place in 15ml tube
* Wash with PBS, aspirate, and trypsinize with 125ul
* Use previously removed media to stop trypsin treatment as well as to break apart clumps of cells
* Spin at 1500 rpm for 10 min
* Aspirate media and re-suspend in 1ml of PBS by GENTLY vortexing
* Transfer to a new 1.5ml tube and spin at 1500rpm for 5 min
* Re-suspend in 1 ml of NIM buffer containing 200ug/ml RNase and 25ug/ml PI
* (optional) Incubate at 30C for 30 minuets
* Incubate for at least 2 hours at 4C in the dark
* Before running samples dilute to less than 5x105 cells/ml – add 0.5 ml of PBS

**FLOW –** Attune Acoustic Focusing Cytometer (Thermo Fisher)

* Run a test sample (NOT RECORD) and adjust forward scatter and side scatter voltage so that the cell population falls in the center of the graph with axis of 8x106
* Run 100,000 events
* Speed – 100 ul/ml
* Acquisition Volume – 450 ul
* Analysis using FlowJo