Protocol: Cell Cycle Analysis/Nuclei

Reagent Preparation-Stock reagents:

1.) 4mM Citrate buffer - 0.588 g Na Citrate in 500 ml dH_2O, pH 7.8
2.) 0.4M NaCL - 11.69 g NaCL in 500 ml dH_2O
3.) RNase Solution: Working RNase Solution should be at 180 units/ml diluted in 1XPBS, and frozen in aliquots at -80^{0}C. (Ribonucleic A, Worthington, Cat. #5679). Store aliquots of RNase at -20°C. (Alternately, 200ug/ml of DNase free RNase A).
4.) 10% TritonX- Add 1ml of Triton X-100 to 9ml of PBS; mix thoroughly.
5.) PI Stock solution: Prepare a 500ug/ml solution of Propidium Iodide.
Note: *Stock reagents can be held at 4°C for up 3 months.*

Reagent Preparation-Staining Solutions:

<u>To prepare 10 ml of *Stain* solution:</u>

1.0ml Propidium Iodide stock solution. (Final concentration 50ug/ml*)
0.5ml RNase stock solution (final concentration 180 units/ml)
0.1ml 10% Triton-X stock solution (final concentration 0.1%)
8.4ml 4mM Citrate Buffer
0.3gm Polyethylene Glycol (PEG)

<u>To prepare 10 ml of Salt solution:</u>

1.0ml P.I. stock solution
0.1ml 10% Triton-X stock solution
8.9ml 0.4 M NaCL solution
0.3g PEG 6000 (*Research Products International Corp. # P48080-1000.0*)

Note: *Staining solutions can be held at 4°C for up to one week. Add fresh RNase prior to using.*

*Final concentration of PI should be established by titration for each test system.

Cell Staining Procedure:

1. Prepare single cell suspension for experiment.
2. Fix cells in ice-cold 70% ethanol and store at -20C until ready to perform assay (minimum 0.5 hour to maximum time of several weeks).
3. Cells should be washed twice in 1ml PBS (add 1 ml PBS, vortex, spin at 1500RPM for five minutes, aspirate supernatant) and reconstituted at $1x10^6$ cells per ml.
4. Centrifuge cell preparation and remove supernatant.

5. Add 1 ml of PI *Stain* solution per $1x10^6$ cells and mix thoroughly. If there are fewer than $1x10^6$ cells, adjust your staining volume accordingly.
6. Incubate at 37°C for 20 minutes.
7. Add PI *Salt* solution in the amount equivalent to stain solution used and mix thoroughly.
8. Store at 4°C in the dark until flow cytometric acquisition and analysis.

References:

Robinson JK, Rademaker AW, Goolsby C, Traczyk TN, Zoladz C. DNA ploidy in nonmelanoma skin cancer. *Cancer,* 15:77(2), 284-91, 1996 Jan 15.

Vindelov, LL, Christensen IJ, Nissen NI. A detergent trypsin method for the preparation of nuclei for flow cytometric DNA analysis. *Cytometry,* 3:323-7, 1983.