DNA CELL CYCLE ANALYSYS USING VINDELOV'S REAGENT
(From Dr. J. B. Turpen's Lab - October 1992)

(NOTE: Vindelov's reagent must be prepared in a plastic container, as RNAase will stick to glass.)

Vindelov's Reagent

100 ml.....Tris Buffered Saline (pH 7.6)
1 mg (350 Units).....Ribonuclease A (Sigma Cat.# R-5000 (Type II-A; 70 U/mg))
7.5 mg .....Propidium Iodide (Sigma Cat.# P-4170)
0.1 ml Nonidet p-40 (Sigma Cat.# N-3516)

Mix in plastic container. Sterile filter and store refrigerated up to 8 weeks (or longer!).

Notes:

1) Store RNAase desicated in refrigerator. (Lot 14F-0266 works well)
2) Shelf life of Vindelov's is long. For publication purposes 8 weeks is very safe, and shelf life is up to 1-2 years.
3) Do not allow reagent to contact glass after addition of RNAase, as RNAase will stick to glass surfaces. (Be careful of pH electrodes!!)

Tris Buffered Saline (pH 7.6)

42 mg .....Tris Base (Trizma Base; Sigma Cat.# T-1503)
58 mg .....NaCl
100 ml.....Distilled H_2O

Mix well. Initial pH will be approximately 9.2, so adjust pH to 7.6 using 1N HCl.

DNA Analysis

Suspend cells in Vindelov's reagent for 1-2 hours prior to analysis. Run samples at slow rate (150 cells/sec or less (slower = better CV's / sharper peaks). Vindelov's creates "bare nuclei", thus the "cells" will have very little Forward Scatter signal. Threshold debris via FwdSctr, then gate using PI-Area vs. PI-Height to eliminate doublets. Viable nuclei will have some FwdSctr and little 90'Sctr. Analyze cell cycle using PI-Area (height works OK too).

Ref: Vindelov, L.L. Flow Microfluorometric Analysis of Nuclear DNA in Cells from Solid Tumors and Cell Suspensions: A New method for Rapid Isolation and Staining of Nuclei. *Virchows Arch.B Cell Path.* 24:227-242, 1977.