Creighton UNIVERSITY

DNA / Cell Cycle Analysis using Vindelov's Reagent

Equipment:

12 x 75 mm test tubes

Reagents:

Vindelov's Reagent

Method:

1) Place 10^6 cells in 12 x 75 mm tube
2) Centrifuge @ 250g for 5 minutes.
3) Discard supernatant and resuspend cells in 1ml Vindelov's reagent.
4) Incubate at 4^oC for 1-2 hours.
5) Analyze on flow cytometer at low flow rate (~150 cells/second or less)

Notes:

1) Samples may need to be filtered through a 30μm mesh to eliminate clumps.
2) Slow data acquisition rate (~150 cells/second or less) results in better CV's for histogram peaks.
3) Vindelov's reagent creates "bare nuclei", thus the "cells" will have little forward scatter signal.
4) Threshold debris using FwdSctr, then gate using PI-area vs. PI-height to eliminate doublets. Viable nuclei will have some FwdSctr and little 90^o-Sctr. Alternatively threshold on PI.
5) Analyze cells using PI-area (or PI-height).

References:

1) Vindelov, LL. *Virchows Arch. B Cell Path*. **24**:227-242, 1977.
2) Krishan, A. *J. Cell Biol.* **66**:188-193, 1975.