Cancer Center
NCI-DESIGNATED COMPREHENSIVE CANCER CENTER

Flow Cytometry Shared Resource (FCSR)
University of Colorado Cancer Center
Anschutz Medical Campus
Aurora, Colorado 80045

KRISHAN STAIN FOR DNA CELL CYCLE ANALYSIS

1. Prepare a single cell suspension with .1 to 1 x 10-6 cells per tube.
2. Wash cells with PBS, then centrifuge to pellet them. Remove the supernatant PBS, vortex to dislodge the pellet, and add 1 ml of Krishan's stain. Vortex again, if clumps are visible, filter the samples immediately. Refrigerate and let stand at least overnight before analysis.

KRISHAN STAIN

Reference: Krishan, A. (1975) J. Cell Biol.; 66,188.

Stain: 0.224 g NaCit.2H2O
9.22 mg Propidium Iodide (carcinogen)
2.0 ml 1% NP-40 Alternative in di or MQ H2O
2.0 ml 1mg/ml RNase, (DNase free)

qs to 200 ml with deionized or MilliQ H2O

Storage: refrigerate
Shelf life: at least 1 year

Flow Cytometric Analysis

Cells are analyzed using a conventional flow cytometer that has a high degree of linearity, such as the Agilent Penteon or Opteon, Bio-rad ZE-5, or Beckman Coulter Gallios. Singlets are excluded using Area vs Height of the Propidium Iodide signal. Cell cycle analysis is completed in FCS Express using the Multi-cycle AV tool

(https://denovosoftware.com/cell-cycle/#:~:text=Cell%20Cycle%20Modeling%20to%20Suit,background%2C%20debris%2C%20and%2For%20aggregation%20correction.)

Supplies from Millipore Sigma (any source should be fine - we have purchased ours from Sigma Aldrich)
Sodium citrate, dihydrate # 567446-100GM
NP-40 Alternative # 492016-100ML
RNase, DNase free # 11119915001
Propidium Iodide # P4170-10MG