ImageStream® Apoptosis with Annexin V and Phi Phi Lux

Unstained and single fluorescent color control samples at 1x10^6 cells per test

Control	Cell type
Unstained	Live
AlexaFluor 647 Annexin V	Apoptotic
PhiPhi Lux Only	Apoptotic
Draq5	Apoptotic

Materials

01. Pacific Blue Annexin V: Invitrogen (Cat#. A35122), 2 mg/mL stock
02. Phi Phi Lux: Oncoimunin (Cat#. A304R1G).
03. DRAQ5: Axxora (cat# BOS-889-001-R200) 5mM Stock
04. Camptothecin (CPT): Sigma (Cat# C-9911, Lot# 022K3446). Make 5 mM 1000X working stock in DMSO, use at 5uM final concentration
05. RPMI 1640, VWR (Cat#. 45000-404) 500ml bottle
06. Annexin V Binding Buffer, 10X: BD Pharmingen (Cat# 556454). Dilute fresh to 2X with dH_2O
07. 2% PFA/Annexin V binding buffer (Fixation Buffer)

Cell preparation

We used Ramos cells cultured in complete RPMI in an incubator containing 5% CO_2 at 37° C. Exponentially growing cells were treated with or without 5 uM CPT for 1-18 hours (at 37°□C under 5% CO_2) to induce apoptosis, at 12 hours >90% are apoptotic. Process cells in 1.5mL microcentrifuge tubes. All washes performed at 300 x g for 5' at 4°C, and cells MUST be mixed gently without vortexing. All stains done at $1x10^7$ cells/ml at 4° C.

01. Harvest cells into 15ml conical tube at 1x10^6th cells per sample, and centrifuge at 300g.
02. Aspirate supernatant and resuspend pellet in 50ul of PhiPhi Lux dilution buffer from the kit.
03. Add 50ul of PhiPhi Lux reagent for a final concentration of about 5uM.
04. Incubate in CO2 incubator at 37° C with caps open for 30 min.
05. Add 100ul of 2x Annexin V binding buffer and add 5ul of Annexin V AF647 and 1ul of 5mM Draq5 (1:200 dilution).
06. Incubate in CO2 incubator at 37° C with caps open for 30 min.
07. Pellet cells at 300g and wash 1x in 1x Annexin V binding buffer.
08. Fix cells with 100ul 2% PFA and 0.5ul Draq5 (1:200 dilution) in 1x Annexin V binding buffer for 15min at RT.
09. Run on ImageStream