40-17. Camptothecin-induced apoptosis Jurkat cells: AnnexinV assay

Experimental Procedures:

Samples (3x10^6 cells per test):

Unstained and single fluorescent color control samples

Control	Cell type
Unstained	Apoptotic
FITC Annexin V	Apoptotic
7-AAD or DRAQ5	Apoptotic

Stain according to following protocol.

Materials

01. FITC annexin V: Bender Med Systems (Cat. BMS306FI), 2 mg/mL stock
02. 7-AAD: Molecular Probes (Cat. A-1310), 1 mM stock
03. DRAQ5: AXXORA LLC, 5 mM stock
04. Camptothecin (CPT): Sigma (Cat# C-9911, Lot# 022K3446) 10 μM in DMSO. Make 1 μM 1000X working stock (good for at least 2 weeks at 4° C) in RPMI.
05. Jurkat cells, clone E6-1: ATCC (TIB-152)
06. RPMI
07. Staining Buffer: 2% FCS/0.1% azide/PBS
08. Annexin V Binding Buffer, 10X: BD Pharmingen (Cat# 556454). Dilute to 1X with dH_2O on day of experiment.
09. 1% PFA/PBS (Fixation Buffer)

Cell preparation

We used Jurkat cells cultured in RPMI supplemented with 5% fetal calf serum in an incubator containing 5% CO_2 at 37° C. Exponentially growing cells were treated with or without 1 μM CPT for 12-18 hours (at 37° C under 5% CO_2) to induce apoptosis. Choice of nuclear probe: 7-AAD stains only late apoptotic and necrotic cells, while DRAQ5 stains all cells. All washes performed at 300 x g for 1o' at 4° C. All stains done at $3x10^7$ cells/ml at 4° C.

01. Wash cells and remove media.
02. Wash once with Stain Buffer
03. Stain with 40 μM 7-AAD (or 50 μM DRAQ5) and a 1:500 dilution of FITC annexin V, and 1X Annexin V Binding Buffer.
04. Wash with Annexin V Binding Buffer.
05. Resuspend at $5x10^7$ cells/ml (75 μL) in 1% PFA/PBS (or if running immediately, in 1X Annexin V Binding Buffer), transfer to 0.6 mL microcentrifuge tubes and run directly on IS100, using 1X Annexin V Binding Buffer as a sheath buffer.