Ernst Lab
2007
C. Jude

Annexin V staining protocol

1. Remove front and rear leg bones remove from all mice, including untreated control.
2. Bones cleaned and crushed in ice cold HBSS + 2% FBS ina motar and pestle.
3. Filter cells through 40µm filter to remove bone fragments into 50 mL conical and pellet at 380 x g for 4 minutes at 4° C.
4. Resuspend in 1mL ACK (red cell lysis buffer) for 5 minutes at room temperature, stop with 5mL HBSS+ pellet at 380 x g for 4 minutes at 4° C.
5. Resuspend in 5mL HBSS+, count 1:20 in trypan blue

Controls:

1. Incubate BM cells from untreated control mouse at $2x10^6$/ ml for 5 minutes at 37^o
    a. DMEM+ (live control)
    b. DMEM+ with 1mM H_2O_2 (apoptotic control) Annexin/PI staining
2. Wash control cells with 5mL HBSS+
3. Filter 10^6 cells/ replicate, stain cell surface antigens as normal
4. Start up flow cytometer and load settings
5. After final wash resuspend controls in appropriate Annexin binding buffer with PI 1µg/mL and/or Annexin V FITC.
6. Set gates for Annexin + cells before beginning staining on experimental samples
7. Resuspend each replicate in 500 µl Annexin Binding buffer with 1:500 Annexin V-FITC and iµg/mL PI
8. Incubate in the dark at room temperature for 15 minutes
9. Analyze on FACSCailbur

Annexin V/ FITC from BD Biosciences

Annexin Binding Buffer (10x stock)

0.1 M Hepes pH 7.4 (make 1M Hepes pH 7.4 by mixing 1M Hepes free acid and 1M Hepes Sodium Salt)

1.4 M NaCl

25mM $CaCl_2$

Dilute to working concentration with H_2O

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Controls

1. Untreated unstained
2. Untreated PI
3. Untreated Annexin V
4. Untreated Annexin V + PI
5. H_2O_2 treated unstained
6. H_2O_2 treated PI
7. H_2O_2 treated Annexin V
8. H_2O_2 treated Annexin V + PI