Annexin V with Cell Surface Antibody Staining for Suspension Cells

Materials and Reagents

Full Name	Short Name	Catalog Number
Microwell plates (round-bottom wells) or tubes (12 x 75-mm polypropylene round-bottom test tube)		N/A
1X PBS Buffer	PBS	554781
BD Pharmingen™ stain buffer, or equivalent, 1X PBS, 2% FBS (or BSA) 0.1% NaN_3 (pH 7.1–7.4)	Stain Buffer (FBS)	554656
	Stain Buffer (BSA)	554657
Annexin V conjugate	Annexin V	*
Propidium Iodide staining solution	PI	556463
BD Via-Probe™ Cell Viability Solution	7-AAD	555816
Annexin V Binding Buffer, 10X Concentrate	Annexin V Binding Buffer	556454

N/A, not applicable
*Select at www.bdbiosciences.com

Procedural Notes

- Investigators might want to check whether the antibody staining procedure affects cell surface levels of Annexin V by including control tubes with or without cell surface antibodies.
- Use PI with FITC-, APC-, or BD Horizon™ V450-conjugated Annexin V; use 7-AAD with PE-, Cy™ 5-, Cy™ 5.5- or BD Horizon™ V500-conjugated Annexin V.
- The following controls are used to set up compensation and quadrants:
  1. Unstained cells
  2. Cells stained with Annexin V-conjugate alone (no viability dye)
  3. Cells stained with viability dye alone (no Annexin V-conjugate)

Procedure

1. Pellet and wash cells using Stain Buffer.
2. Optional: cells may be stained with antibodies against cell surface antigens prior to the Annexin V staining procedure. If you are not staining cells with antibodies, proceed to step 3.
   i. Wash cells with Stain Buffer and resuspend in 100 µL in 12 x 75-mm tubes or a 96-well plate.
   ii. Add antibodies against cell surface antigens and incubate for 20-45 minutes in the dark (either on ice or at RT).
   iii. Wash cells twice (1-2 mL for tubes or 100-200 µL for 96-well plates, centrifuging at 300*g*) with Stain Buffer, then proceed with step 3 below.
3. Wash cells twice with cold PBS and then resuspend cells in 1X Binding Buffer at a concentration of ~1 x 10^6 cells/mL.
4. Transfer 100 µL of the solution (~1 x 10^5 cells) to a 5-mL culture tube.
5. Add Annexin V and PI or 7-AAD as described in the Technical Data Sheet or the Annexin V apoptosis detection kit manual.
6. Gently mix the cells and incubate for 15 minutes at RT in the dark.

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7. Add 400 µL of 1X Binding Buffer to each tube. Analyze by flow cytometry as soon as possible (within 1 hr).

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