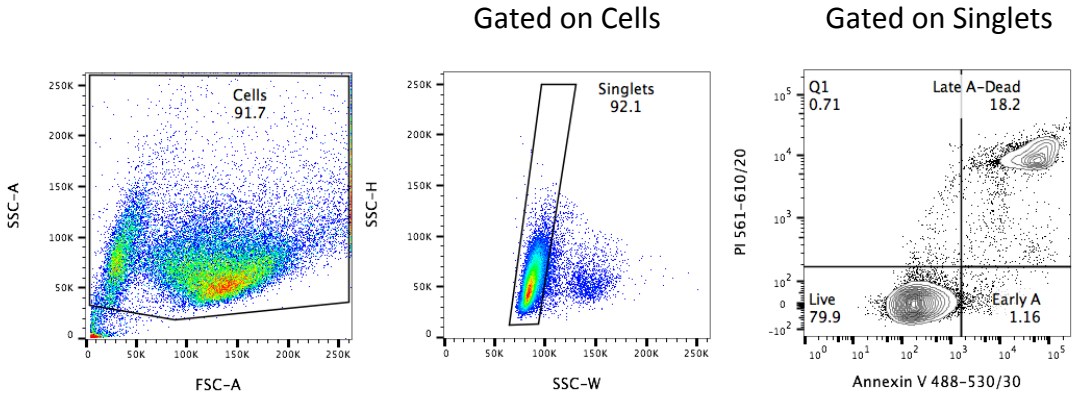
Apotosis determination

(Annexin v-Propidium Iodide)

Apoptosis is a normal physiologic process, which occurs during embryonic development and maintenance of tissue homeostasis. The apoptotic program is characterized by certain morphologic features, including loss of plasma membrane.

In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. Annexin V is a 35-36 kDa Ca2+ dependent phospholipid-binding protein that has a high affinity for PS, and binds to cells with exposed PS. Annexin V may be conjugated to fluorochromes, and used in flow cytometric analysis to detect cells that are in early stages of apoptosis.

Staining with Annexin V is typically used in conjunction with a vital dye such as Propidium iodide (PI) or 7-Amino-Actinomycin (7-AAD) to allow the investigator to identify: early apoptotic cells (PI negative, Annexin V positive), late apoptosis/dead (Annexin V and PI positive), while viable cells with intact membranes appears as Annexin V and PI negative.



Data from Johannes Popow/Hentze Group.

Protocol

1. Recover cells from the tissue or culture plate
2. Wash cells twice with cold PBS 1X and then resuspend 1x106 cells in 1ml of Annexin V binding buffer
3. Transfer 100µl of the cell suspension in a new tube
4. Add 5µl of Annexin V

5. Add 5µl of PI (1mg/ml)
6. Gently vortex the cells and incubate for 15 min at RT in the dark
7. Add 400µl of 1X Binding Buffer to each tube. Analyze by flow cytometry within 1 hr.

*When using an adherent cell line, recover the supernatant of the culture before starting the tripsine treatment. Apoptotic cells migh be present in this fraction.

References

Protocol slightly modified from Technical Data Sheet of FITC Annexin V reagent. BD Pharmingen™