Annexin V CoraLite® 488 and PI

Instructions

The following experimental protocol has been optimized using staurosporine to induce apoptosis in Jurkat cells. Some modifications may be required if other inducers or cell types are used.

Flow cytometry

1. **Induce cell apoptosis in accordance with your experimental protocol. Untreated cell samples should also be included as a negative control. Appropriate samples for single dye staining and compensation should also be included.**

2. **Centrifuge your cell suspension at 300 x g's for 5 minutes at 4 °C to pellet your cells. For adherent cells: first perform digestion with EDTA-free trypsin, then centrifuge at 300 x g's for 5 min at 4 °C to collect cells: trypsinization time should not be too long to prevent false positives.**

   Note: After digestion with trypsin, incubate cells in optimal cell culture conditions and medium for about 30 minutes before staining. Trypsin digestion temporarily destroys the plasma membrane, allowing Annexin V to bind phosphatidylserine on the cytoplasmic surface of the cell membrane, resulting in false positive staining.

3. **Wash cells twice with ice cold PBS and centrifuge at 300 x g's for 5 min at 4 °C each time, collect 1-5×10^5 cells and resuspend in 100 µL of 1× binding buffer.**

4. **Add 4-5 µL of CL488-Annexin V and 5 µL of PI working solution to each tube.**
   Note: We recommend preparing two additional flow tubes for controls. Add a single dye, CL488-Annexin V or PI, to each tube to calculate compensation adjustment for each of the dyes.

5. **Incubate for 10-15 minutes at room temperature in the dark.**

6. **After the incubation period, add 400 µL of 1 X binding buffer (or 1x PBS) ito each tube. As soon as possible, analyze the stained cells on a flow cytometer. CL488-Annexin V is excited by a 488 nm laser, and the detection fluorescence emission spectrum is at 530 nm (FITC channel), and the emission spectrum of PI is around 617 nm.**
Note: The choice of PBS or 1X binding buffer depends on the specific selection of different apoptosis treatments and different cells.

Fluorescence microscopy

For cells in suspension, refer to the flow cytometry method for specific instructions.

1. **Deposit cells onto a glass slide or culture plate at an appropriate density of ~1 x 10^6 cells/mL.**

2. **Induce cell apoptosis according to experimental requirements. One sample should contain untreated cells as a negative control.**

3. **Wash the cells with PBS.**
Note: If cells are not washed with PBS after cell collection, serum-containing medium can be used to directly replace Annexin V binding buffer, but the concentration of Annexin V needs to be re-optimized.

4. **Create a working stock by adding 5-25 µL of CL488-Annexin V and 5 µL of PI to every 100 µL of 1 X Annexin V binding buffer.**
Note: The optimal use concentration is determined by specific experimental requirements.

5. **Add enough dye solution to the culture plate or glass slide to cover all cells, and incubate for 15-30 min at room temperature in the dark.**

6. **Wash the cells with 1X binding buffer.**

7. **Mount the slides using your desired method. Alternatively, if using a culture plate, add sufficient 1x binding buffer to cover the cells.**

8. **Use a fluorescent microscope to image the cells. For detecting CL488-Annexin V, use filters suitable for FITC, and for PI, use filters suitable for Cy3 or Texas Red.**