Hoechst 33342/Propidium Iodide Apoptosis Assay Kit

Cat. No. CTG-AP0008 Store the kit at -15 to -25°C

Experimental Protocol

This assay has been optimized by using Jurkat cells treated with camptothecin to induce apoptosis. Some modifications may be required for use with other cell types.

1. Induce apoptosis in cells using the desired method. A negative control should be prepared by incubating cells in the absence of inducing agent.
2. Harvest the cells after the incubation period, wash in cold phosphate-buffered saline (PBS) and adjust the cell density to ~1 × 10^6 cells/mL in PBS. For each assay a 1 mL volume will be used.
3. Add 1 µL of the Hoechst 33342 stock solution (Component A) and 1 µL of the PI stock solution (Component B) to each 1 mL of cell suspension.
4. Incubate the cells on ice for 20-30 minutes.
5. As soon as possible after the incubation period, analyze the stained cells by flow cytometry, using UV/488 nm dual excitaiton and measuring the fluorescence emission at ~460 nm and >575 nm. The population should separate into three groups: live cells will show only a low level of fluorescence, apoptotic cells will show a higher level of blue fluorescence and necrotic cells will show both blue and red fluorescence.

References

1. Cytometry 27, 1 (1997);
2. Immunol Cell Biol 76, 1 (1998);
3. J Pharmacol Toxicol Methods 37, 215 (1997);
4. FASEB J 9, 1277 (1995);
5. Am J Pathol 146, 3 (1995);
6. Cytometry 17, 59 (1994);