Apoptosis

APOPTOSIS or programmed cell death is marked by a series of characteristics including loss of cell volume, zeiosis, clumping of chromatin and nuclear fragmentation into apoptotic bodies. There are several flow cytometric-based methods that can be used to quantitate apoptosis by flow cytometry.

Sub-Diploid (Ao) Population Detection

One of the simplest methods is to use propidium iodide to stain the DNA and look for the sub-diploid, or Ao, population of cells from a cell cycle profile. The most commonly used dye for DNA content/cell cycle analysis is propidium iodide (PI). The PI intercalates into the major groove of double-stranded DNA and produces a highly fluorescent adduct that can be excited at 488 nm with a broad emission centered around 600 nm. Since PI can also bind to double-stranded RNA, it is necessary to treat the cells with RNase for optimal DNA resolution.

The excitation of PI at 488 nm facilitates its use on the benchtop cytomters. [PI can also be excited in the UV (351-364 nm line from the argon laser) which should be considered when performing multicolor analysis on the multibeam cell sorters].

TUNEL Assay

TUNEL assay measures DNA strand breaks and annexin V binding, which detects relocation of membrane phosphatidyl serine from the intracellular surface to the extracellular surface.

CASPASE Activity

CASPASE activity (cysteine proteases), typically CASPASE-3, can be detected using a fluorogenic substrate kit. Microscopic examination and detection of DNA laddering by gel electrophoresis will be used to confirm the flow cytometric results.