

2 Apoptosis Assay Methods

Originally, to study both forms of cell death, necrosis and apoptosis, cytotoxicity assays were used. These assays were principally of two types:

- ▶ Radioactive and non-radioactive assays that measure increases in plasma membrane permeability, since dying cells become leaky.
- ▶ Colorimetric assays that measure reduction in the metabolic activity of mitochondria; mitochondria in dead cells cannot metabolize dyes, while mitochondria in live cells can.

Note: For a detailed discussion of both types of cytotoxicity assay, see Section A 3, beginning on page 58 of this guide.

However, as more information on apoptosis became available, researchers realized that both types of cytotoxicity assays vastly underestimated the extent and timing of apoptosis. For instance, early phases of apoptosis do not affect membrane permeability, nor do they alter mitochondrial activity. Although the cytotoxicity assays might be suitable for detecting the later stages of apoptosis, other assays were needed to detect the early events of apoptosis.

In concert with increased understanding of the physiological events that occur during apoptosis, a number of assay methods have been developed for its detection. For instance, these assays can measure one of the following apoptotic parameters:

- ▶ Fragmentation of DNA in populations of cells or in individual cells, in which apoptotic DNA breaks into different length pieces.
- ▶ Alterations in membrane asymmetry. Phosphatidylserine translocates from the cytoplasmic to the extracellular side of the cell membrane.
- ▶ Activation of apoptotic caspases. This family of proteases sets off a cascade of events that disable a multitude of cell functions.
- ▶ Release of cytochrome C and AIF into cytoplasm by mitochondria.

For practical reasons, we have divided this chapter into two broad categories: assays that measure apoptosis in cell populations (Section A 2.1 of this guide) and assays that measure apoptosis in individual cells (Section A 2.2 of this guide).

For discussions of particular assays, turn to the pages indicated in the product selection guide (Figure 3).

