

2. Special applications of cell death and cell proliferation methods

This section of the Appendix contains condensed versions of articles that appeared in the Roche Applied Science Biochemica newsletter. For further experimental detail and background, see the full *Biochemica* articles.

2.1 TUNEL assays

2.1.1 Discrimination between dead and viable apoptotic cells using two-color TdT assay and surface labeling as detected by flow cytometry

[from Earl A. Timm, Jr. and Carleton C. Stewart, Laboratory of Flow Cytometry, Roswell Park Cancer Institute, Buffalo, N.Y., USA]

Note: This article appeared in Biochemica No. 1 (1996), 44–47.

Summary: The TUNEL method uses terminal dideoxynucleotidyl transferase (TdT) to incorporate hapten-tagged nucleotides into the 3'-strand breaks that occur in DNA during apoptosis (Gorczyca et al., 1993; Chapman et al., 1995). If these nucleotides are coupled to a fluorescent molecule, or if the hapten can be detected by a fluorescent secondary reagent, the apoptotic cells can be analyzed by flow cytometry.

Flow cytometry permits not only the detection of apoptotic populations, but also the simultaneous detection and immunophenotyping of necrotic populations. The drawback to using the current TdT method, however, is that the ethanol permeabilization of the cells is incompatible with immunophenotyping because it denatures cellular epitopes (Darzynkiewicz et al., 1992; Li et al., 1995).

A protocol has been developed that both preserves the surface markers and detects apoptotic cells. In addition, it is possible to discriminate between dead apoptotic cells and viable apoptotic cells with a second hapten-tagged nucleotide that labels dead cells. The method also can distinguish dead apoptotic cells from cells that have died by other mechanisms (e.g., necrosis).

2.1.2 The use of flow cytometry for concomitant detection of apoptosis and cell cycle analysis

[from E. Hanon, A. Vanderplasschen and P.-P. Pastoret, Department of Immunology/Vaccinology, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium]

Note: This article appeared in Biochemica No. 2 (1996), 25–27.

Summary: Two distinct modes of cell death, apoptosis and necrosis, can be distinguished on the basis of differences in morphological, biochemical, and molecular changes occurring in the dying cells (Duvall and Wyllie, 1986).

Cells undergoing apoptosis display a characteristic pattern of structural changes in the nucleus and cytoplasm, including rapid blebbing of the plasma membrane and nuclear disintegration (Duvall and Wyllie, 1986). Extensive damage to chromatin and cleavage of DNA into oligonucleosomal-length fragments both occur during apoptosis (Duvall and Wyllie, 1986).

Several flow cytometric methods for identifying cells undergoing DNA fragmentation have been described recently. These include DNA content analysis and *in situ* labeling of DNA fragments with tracer-dUTP. The former is based on the accumulation of ethanol-

