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Apoptosis : a practical approach /

Studzinski, George P.

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Laboratory Manual | Laboratory manuals. | Laboratory manuals. | Manuels de laboratoire.

Monografía

This text is designed to provide conceptual outlines and detailed procedures for basic and advanced studies of cell death by apoptosis. Chapters on the recognition of apoptosis as distinguished from necrosis and nonspecific cell DNA damage, are followed by a systematic examination of the established and the principal novel methodologies utilized by some leading laboratories conducting research on apoptosis. The organization is on the lines of signalling for apoptosis, the apoptotic cascade, and the execution of apoptosis. A wide variety of procedures are provided which will enable the reader to participate in cutting-edge research

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Contenido: Intro; Contents; List of Contributors; Abbreviations; 1. Overview of apoptosis; 1. General introduction and overview of contents; 2. Historical perspective; 3. Distinction of apoptosis from other forms of cell death; 4. Apoptotic cascades; 5. Time course of apoptotic cascades; 6. Selection of methods; Procedure for determination of increased mitochondrial to nuclear DNA ratio, for the detection and quantitation of apoptosis; 7. Pitfalls; References; 2. Morphological recognition of apoptotic cells; 1. Introduction; Key morphological features of apoptotic cells 2. Light and fluorescent microscopy techniques for the assessment of apoptosisPreparation of cell or tissue samples; Nuclear counterstains; 3. Electron microscopic techniques; 4. Quantitation of apoptotic events; Methods; Problems in scoring apoptotic events; 5. In situ detection of DNA strand breaks; 6. Other techniques; 7. Conclusions; References; 3. Assessment of DNA damage in apoptosis; 1. Introduction; 2. Types of DNA fragmentation; 3. Measurement of high molecular weight DNA fragmentation by pulse-field gel electrophoresis; Pulse-field gel electrophoresis procedures Strategies for electrophoretic separations4. Detection of internucleosomal DNA fragmentation by standard agarose gel electrophoresis; 5. Filter elution assay to measure apoptotic DNA fragmentation; Equipment; Filters and solutions; DNA labelling and preparation of experimental

cell cultures; Protocols; Counting samples and computations; References; 4. Analysis of cell death by flow and laser-scanning cytometry; 1. Introduction; 2. Preparation of cells for analysis by LSC. Detection of apoptotic cells based on changes in nuclear chromatin Fas-based cytotoxicityPerforin/granzyme-based cytotoxicity; 2. Standard methods for the evaluation of cell-mediated cytotoxicity; Chromium release assay; [[sup(125)]I]Iododeoxyuridine ([sup(125)]IUdR) release assay; The JAM test; BLT-esterase release assay; Estimation of target cell nuclear fragmentation using fluorescent dyes; Activation-induced cell death (AICD); 3. Separate studies of Fas- and perforin/granzyme-based cytotoxicity; Fas-based cytotoxicity in the absence of perforin/granzyme contribution; Perforin/granzyme-based cytotoxicity in the absence of FasL contribution

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Autores: Studzinski, George P.

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Baratz Innovación Documental

- Gran Vía, 59 28013 Madrid
- (+34) 91 456 03 60
- informa@baratz.es