CONTENTS

Contributors	
Preface	xv
1. Isolation and Assay of Caspases	
Srinivasa M. Srinivasula, Ayman Saleh, Manzoor Ahmad, Teresa Fernandes-Alnemri, and Emad S. Alnemri	
I. Introduction	1 5
II. Materials Required III. Methods	5 6
References	25
2. Cloning and Analysis of Bcl-2 Family Genes	
- ,	
Enrique Cepero, Bryan W. Johnson, and Lawrence H. Boise	
I. Introduction	29
II. Cloning of Bcl-2 Family Genes	33
III. Analysis of Bcl-2 Family Protein Function and Regulation References	36
References	. 45
3. Flow Cytometric Analysis of Cell Shrinkage and Monovalent Ions during Apoptosis	
Carl D. Bortner and John A. Cidlowski	
I. Introduction	49
II. Analysis of Cell Size by Flow Cytometry	52
III. Analysis of Monovalent Intracellular Ions by Flow Cytometry	57
IV. Sorting Apoptotic Cells by Flow Cytometry	64
References	67
4. Use of Flow and Laser-Scanning Cytometry in Analysis of Cell Death	
Zbigniew Darzynkiewicz, Xun Li, and Elzbieta Bedner	
I. Introduction	70
II. Principles of Cell Measurement by Laser-Scanning Cytometry (LSC)	72
III. Specifics of Cell Preparation for Analysis by LSC	75
IV. Chromatin Condensation as a Marker of Apoptotic Cells Detected by LSC	77
V. Gross Changes in Cell Structure during Apoptosis Measured	
by Laser-Light Scattering	79

	te	

	VI. Dissipation of Mitochondrial Transmembrane Potential $(\Delta\Psi_{m})$	
	during Apoptosis	80
	VII. Annexin V as a Marker of Apoptotic Cells	84
	VIII. DNA Fragmentation Assay: Detection of Cells with Fractional ("Subdiploid")	87
	DNA Content IV DNA For any action Detection of DNA Second Breaks ("TI INEL" Asset)	90
	IX. DNA Fragmentation: Detection of DNA Strand Breaks ("TUNEL" Assay) X. Analysis of Caspase Activation by the Labeled Inhibitor Method	93
	XI. Detection of Apoptotic Cells Based on Cleavage of Poly(ADP-ribose)	75
	Polymerase	97
	XII. Unique Possibilities Offered by LSC in Studies of Apoptosis	100
	XIII. Strategies in Analysis of Apoptosis by Flow Cytometry or LSC	102
	References	105
5.	Analysis of Protein Transglutamylation in Apoptosis	
	Zoltán Nemes, András Mádi, Lyuben N. Marekov, Mauro Piacentini, Peter M. Steinert,	
	and László Fésüs	
	I. Introduction	112
	II. Transglutaminase (TGase) Activity Assays	114
	III. Determination of ε-(γ-Glutamyl)-lysine Cross-Link Density	
	in Apoptotic Tissue	115
	IV. Immunoprecipitation of TGase-Interacting Proteins from Apoptotic Cells	119
	V. In Situ Identification of TGase Glutamyl Donor Substrate Proteins	
	in Apoptotic Tissue	119
	VI. Identification of Labeled Substrate Proteins	124
	VII. Identification of TGase Reactive Glutamine Residues by	
	Protein Sequencing	125
	VIII. Location of Cross-Links in Protein Complexes	126
	IX. Analysis of TGase-Mediated Glutamine Deamidation by	
	Peptide Sequencing	130
	X. Reagents	131
	References	132
6.	Analysis of Sphingomyelin and Ceramide Levels and the Enzymes	
٠.	Regulating their Metabolism in Response to Cell Stress	
	Rick T. Dobrowsky and Richard N. Kolesnick	
	I. Introduction	136
	II. Metabolic Labeling	136
	III. Determination of Sphingomyelin Mass Levels	142
	IV. Enzymatic Method for the Quantitation of Ceramide-Diacylglycerol (DAG)	
	Kinase Assay	144
	V. Chromatographic Quantitation of Ceramide Using HPLC and	
	Fluorescence Spectroscopy VI. Assays for Enzymes Regulating Cellular Levels of Sphingomyelin	147
	and Ceramide	140
	References	149

7.	Cell-Free Systems to Study Apoptosis Howard O. Fearnhead	
	I. Introduction II. Preparation of Extracts III. In Vitro Apoptosis Assays IV. Factors That Affect Caspase Activation in Extracts V. Concluding Remarks References	167 168 171 181 183 183
8.	Role of c-Jun N-terminal Kinase in Apoptosis	
	Zheng-gang Liu, Joseph Lewis, Tzu-Hao Wang, and Amy Cook	
	 I. Introduction II. Methods for the Detection of c-Jun N-terminal Kinase (JNK) Activation during Apoptosis III. Evaluation of the Role of JNK Activation in Apoptosis References 	190 193 193
9.	Methods for Studying Pro- and Antiapoptotic Genes in Nonimmortal Cells	
	Mila E. McCurrach and Scott W. Lowe	
	 I. Introduction II. Mouse Embryo Fibroblasts (MEFs) III. Retroviral-Mediated Gene Transfer IV. Basic Infection Protocol for MEFs (Using the Phoenix System) V. Analysis of Cells VI. Other Systems References 	198 199 202 205 210 222 225
10.	Calcium Flux Measurements in Apoptosis	
	David J. McConkey and Leta K. Nutt	
	 I. Background II. Ca²⁺-Specific Probes III. Calcium Imaging IV. Summary and Conclusions References 	229 234 237 242 243
11.	Proteinase Families and Their Inhibitors Donald L. Mykles	
	 I. Introduction II. Enzyme Classification III. Proteinase Inhibitors IV. Considerations for Inhibitor Selection and Application V. General Guidelines for Using Proteinase Inhibitors 	247 248 249 259 265

		Contents
	VI. Concluding Remarks	266
	References	267
12.	Identification and Analysis of Caspase Substrates: Proteolytic Cleavage of Poly(ADP-ribose)polymerase and DNA Fragmentation Factor 45	
	Claudia Boucher, Stéphane Gobeil, Kumiko Samejima, William C. Earnshaw, and Guy G. Poirier	
	I. Introduction	290
	II. Poly(ADP-ribose)polymerase (PARP-1)	291
	III. DNA Fragmentation Factor II (DFF45)	300
	References	303
13.	Analysis of Reactive Oxygen Species in Cell Death	
	Ivan Stamenkovic	
	I. Introduction	307
	II. Methods	309
	References	318
14.	Methods for Studying Apoptosis and Phagocytosis of Apoptotic Cells in <i>Drosophila</i> Tissues and Cell Lines	
	Kristin White, Simonetta Lisi, Phani Kurada, Nathalie Franc, and Peter Bangs	
	I. Introduction	321
	II. Detection of Apoptosis in Drosophila Tissues	323
	III. Phagocytosis	330
	IV. Testing for Apoptotic Activities in Drosophila S2 Cells	332
	References	338
15.	Phosphatidylserine Exposure and Phagocytosis of Apoptotic Cells	
	Patrick Williamson, Stefan van den Eijnde, and Robert A. Schlegel	
	I. Introduction	340
	II. Annexin V Purification	342
	III. Annexin Staining for Flow Cytometric Analysis of Apoptotic Cells	346
	IV. In Vivo Detection of Cell Surface-Exposed PS	349
	V. MC540 Staining	354 355
	VI. Transbilayer Lipid Movement Assays VII. Phagocytosis of Apoptotic Cells	358
	References	362
16	. The (Holey) Study of Mitochrondria in Apoptosis	
	Nigel J. Waterhouse, Joshua C. Goldstein, Ruth M. Kluck, Don D. Newmeyer, and Douglas R. Green	
	I Introduction	366
	 II. Induction of Cytochrome ε Release from Mitochrondria in Cultured Cells III. Assessment of Cytochrome ε Release in Heterogeneous Populations 	368
	of Cultured Cells	369

Contents	ix
IV. Assessment of Cytochrome c Release in Single Cells	372
V. Assessment of Mitochondrial Cytochrome ε Release in Cell-Free Systems	375
VI. Detection of Proteins Released from Mitochondria Concomitant with	277
Cytochrome c VII. Assessment of Mitochondrial Function	377 379
References	386
Appendix	390
17. In Situ Detection of Dying Cells in Normal and Pathological Tissues	
Christos Valavanis, Stephen Naber, and Lawrence M. Schwartz	
I. Introduction	394
II. Morphological Features of Cell Death: Apoptosis vs Necrosis	395
III. Methods for Apoptotic Cell Identification	397
IV. TUNEL V. ISEL	397 402
VI. Comments for Both TUNEL and ISEL	402
VII. Other Methods	406
VIII. General Considerations	410
References	412
18. Model Cell Lines for the Study of Apoptosis in Vitro	
Christos Valavanis, Yanhui Hu, Yili Yang, Barbara A. Osborne, Salem Chouaib, Lloyd Greene, Jonathan D. Ashwell, and Lawrence M. Schwartz	
I. Introduction	418
II. Jurkat	424
III. 2B4.11	426
IV. DO11.10	427
V. MCF-7	428
VI. PC12	429
VII. C_2C_{12}	430
References	431
19. Programmed Cell Death Assays for Plants	
Alan M. Jones, Silvia Coimbra, Angelika Fath, Mariana Sottomayor, and Howard Thomas	
I. Introduction	438
II. DNA Fragmentation by Gel Electrophoresis	438
III. TUNEL	443
IV. Nondestructive Measurements of Plant Cell Senescence	447
References	450
20. Studies of Apoptosis Proteins in Yeast	
Hong Zhang and John C. Reed	
I. Introduction	453
II. Methods	456

III. Inhibition of Caspase- and Bax-Induced Cell Death in Yeast

465

		Contents
	IV. Comments and Conclusions	466
	References	467
21.	Methods to Study Cell Death in Dictyostelium discoideum	
	Jean-Pierre Levraud, Myriam Adam, Sophie Cornillon, and Pierre Golstein	
	I. Introduction	470
	II. Cell Culture and Maintenance of Axenic Strains	473
	III. Induction of Developmental Cell Death	476
	IV. Analysis of Cell Death Characteristics	480
	V. Genetic Manipulation of Dictyostelium	485
	VI. Selection and Analysis of Cell Death-Resistant Mutants	491
	References	494
22.	Methods of Study of Tumor Necrosis Factor-Related Ligands in Apoptosi	s
	Isabelle A. Rooney, Chris A. Benedict, Paula S. Norris, and Carl F. Ware	
	I. Introduction	500
	II. Production and Characterization of Reagents	503
	III. Receptor Binding Characteristics of TNF Family Ligands	512
	IV. Induction of Apoptosis	517
	V. Effect of Viral Infection on Cell Susceptibility to TNF Ligand-Mediated	
	Cytotoxicity	520
	References	524
Inde	Ar.	527
Volu	imes in Series	547