

Methods in Enzymology

Volume 322

Apoptosis

EDITED BY

John C. Reed

THE BURNHAM INSTITUTE
LA JOLLA, CALIFORNIA



ACADEMIC PRESS

San Diego London Boston New York Sydney Tokyo Toronto

Table of Contents

CONTRIBUTORS TO VOLUME 322	ix
PREFACE	xiii
VOLUMES IN SERIES	xv

Section I. Measuring Apoptosis and Apoptosis-Induced Endonucleases

1. Detection of DNA Cleavage in Apoptotic Cells	SCOTT H. KAUFMANN, PETER W. MESNER, JR., KUMIKO SAMEJIMA, SHIGENOBU TONÉ, AND WILLIAM C. EARNSHAW	3
2. Detection of Apoptosis by Annexin V Labeling	ELLA BOSSY-WETZEL AND DOUGLAS R. GREEN	15
3. Analysis of Apoptotic Cells by Flow and Laser Scanning Cytometry	ZBIGNIEW DARZYNKIEWICZ AND ELZBIETA BEDNER	18
4. Quantitative Measurement of Apoptosis Induced by Cytotoxic T Lymphocytes	MICHELE BARRY, JEFFREY HEIBEIN, MICHAEL PINKOSKI, AND R. CHRIS BLEACKLEY	40
5. Apoptotic Nuclease Assays	FRANCIS M. HUGHES, JR., AND JOHN A. CIDLOWSKI	47

Section II. Measuring Apoptosis in Lower Organisms

6. Analysis of Programmed Cell Death and Apoptosis in <i>Drosophila</i>	PO CHEN AND JOHN M. ABRAMS	65
7. Programmed Cell Death in the Nematode <i>Caenorhabditis elegans</i>	DUNCAN LEDWICH, YI-CHUN WU, MONICA DRISCOLL, AND DING XUE	76

Section III. Proteases Involved in Apoptosis and Their Inhibitors

8. Caspase Assays	HENNING R. STENNICKE AND GUY S. SALVESEN	91
-------------------	---	----

9. Determination of Caspase Specificities Using a Peptide Combinatorial Library	NANCY A. THORNBERRY, KEVIN T. CHAPMAN, AND DONALD W. NICHOLSON	100
10. Criteria for Identifying Authentic Caspase Substrates during Apoptosis	SOPHIE ROY AND DONALD W. NICHOLSON	110
11. Purification and Use of Granzyme B	LIANFA SHI, XIAOHE YANG, CHRISTOPHER J. FROELICH, AND ARNOLD H. GREENBURG	125
12. Viral Caspase Inhibitors CrmA and p35	QIAO ZHOU AND GUY S. SALVESEN	143
13. Purification and Use of Recombinant Inhibitor of Apoptosis Proteins as Caspase Inhibitors	QUINN L. DEVERAUX, KATE WELSH, AND JOHN C. REED	154
14. Monitoring Activity of Caspases and Their Regulators in Yeast <i>Saccharomyces cerevisiae</i>	CHRISTINE J. HAWKINS, SUSAN L. WANG, AND BRUCE A. HAY	162

Section IV. Cell-Free Systems for Monitoring Steps in Apoptosis Pathways

15. <i>In Vitro</i> Assays for Caspase-3 Activation and DNA Fragmentation	XUESONG LIU AND XIAODONG WANG	177
16. Cell-Free Apoptosis in <i>Xenopus laevis</i> Egg Extracts	OLIVER VON AHSEN AND DONALD D. NEWMAYER	183
17. Cytofluorometric Quantification of Nuclear Apoptosis Induced in a Cell-Free System	HANS K. LORENZO, SANTOS A. SUSIN, AND GUIDO KROEMER	198

Section V. Mitochondria and Apoptosis

18. Purification of Mitochondria for Apoptosis Assays	SANTOS A. SUSIN, NATHANAEL LAROCHELLE, MAURICE GEUSKENS, AND GUIDO KROEMER	205
19. Quantitation of Mitochondrial Transmembrane Potential in Cells and in Isolated Mitochondria	NAOUFAL ZAMZAMI, DIDIER MÉTIVIER, AND GUIDO KROEMER	208
20. Nitrogen Cavitation for Cell Disruption to Obtain Mitochondria from Cultured Cells	ROBERTA A. GOTTLIEB AND SOUICHI ADACHI	213

21. Apoptosis-Related Activities Measured with Isolated Mitochondria and Digitonin-Permeabilized Cells	GARY FISKUM, ALICIA J. KOWALTOWSKI, ALEXANDER Y. ANDREYEV, YULIA E. KUSHNAREVA, AND ANATOLY A. STARKOV	222
22. Assays for Cytochrome <i>c</i> Release from Mitochondria during Apoptosis	ELLA BOSSY-WETZEL AND DOUGLAS R. GREEN	235
23. Purification and Liposomal Reconstitution of Permeability Transition Pore Complex	CATHERINE BRENNER, ISABEL MARZO, HELENA L. DE ARAUJO VIEIRA, AND GUIDO KROEMER	243

Section VI. Bcl-2 Family Proteins

24. Monitoring Interactions of Bcl-2 Family Proteins in 96-Well Plate Assays	JOSÉ-LUIS DIAZ, TILMAN OLTERSDORF, AND LAWRENCE C. FRITZ	255
25. Analysis of Dimerization of Bcl-2 Family Proteins by Surface Plasmon Resonance	ZHIHUA XIE AND JOHN C. REED	266
26. Measuring Pore Formation by Bcl-2 Family Proteins	SHARON L. SCHENDEL AND JOHN C. REED	274
27. Assays for Studying Bax-Induced Lethality in the Yeast <i>Saccharomyces cerevisiae</i>	QUNLI XU, NING KE, SHIGEMI MATSUYAMA, AND JOHN C. REED	283
28. Exploiting the Utility of Yeast in the Context of Programmed Cell Death	CATHERINE N. TORGLER, ROBIN BROWN, AND ERIC MELDRUM	297

Section VII. Studying Receptors and Signal Transduction Events Implicated in Cell Survival and Cell Death

29. Production of Recombinant TRAIL and TRAIL Receptor:Fc Chimeric Proteins	PASCAL SCHNEIDER	325
30. Expression of Lymphotoxins and Their Receptor-Fc Fusion Proteins by Baculovirus	ISABELLE ROONEY, KRISTINE BUTROVICH, AND CARL F. WARE	345
31. Analysis of the CD95 (APO-1/Fas) Death-Inducing Signaling Complex by High-Resolution Two-Dimensional Gel Electrophoresis	CARSTEN SCAFFIDI, FRANK C. KISCHKEL, PETER H. KRAMMER, AND MARCUS E. PETER	363

32. Measurement of Ceramide Levels by the Diacyl- glycerol Kinase Reaction and by High-Perfor- mance Liquid Chromatography-Fluorescence Spectrometry	RON BOSE AND RICHARD KOLESNICK	373
33. Measurement of Ceramide Synthase Activity	RON BOSE AND RICHARD KOLESNICK	378
34. Measurement of Sphingomyelinase Activity	ERICH GULBINS AND RICHARD KOLESNICK	382
35. Assays for JNK and p38 Mitogen-Activated Pro- tein Kinases	TATSUHIKO SUDO AND MICHAEL KARIN	388
36. Assaying for I _K B Kinase Activity	JOSEPH A. DiDONATO	393
37. Assays for Akt	THOMAS F. FRANKE	400

Section VIII. Other Methods

38. Measurement of Cellular Oxidation, Reactive Oxy- gen Species, and Antioxidant Enzymes during Apoptosis	LISA M. ELLERBY AND DALE E. BREDESEN	413
39. Volume Regulation and Ion Transport during Apoptosis	CARL D. BORTNER AND JOHN A. CIDLOWSKI	421
40. Assays for Transglutaminases in Cell Death	GERRY MELINO, ELEONORA CANDI, AND PETER M. STEINERT	433
41. Anoikis	STEVEN M. FRISCH	472
42. Transient Transfection Assay of Cell Death Genes	MASAYUKI MIURA AND JUNYING YUAN	480
43. Sindbis Virus Vector System for Functional Analy- sis of Apoptosis Regulators	J. MARIE HARDWICK AND BETH LEVINE	492
44. Transduction of Full-Length Tat Fusion Proteins Directly into Mammalian Cells: Analysis of T Cell Receptor Activation-Induced Cell Death	ADAMINA VOCERO-AKBANI, NATALIE A. LISSY, AND STEVEN F. DOWDY	508
AUTHOR INDEX		523
SUBJECT INDEX		555