

Laboratory Methods in Cell Biology

Biochemistry and Cell Culture

Volume 112

P. Michael Conn



ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON
NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

Academic Press is an imprint of Elsevier



Contents

Contributors	xi
Preface: Laboratory Methods in Cell Biology	xix
CHAPTER 1 Extraction/Characterization of DNA	1
Leland J. Cseke, Joseph R. Herdy	
1 Purpose	2
2 Theory	2
3 Equipment (in order of appearance)	2
4 Materials (in order of appearance)	3
5 Protocol	6
CHAPTER 2 Co-Immunoprecipitation: Isolation of Protein Signaling Complexes from Native Tissues	33
Adele Stewart, Rory A. Fisher	
1 Purpose	34
2 Theory	34
3 Equipment	36
4 Materials	36
5 Protocol	38
CHAPTER 3 Real-Time PCR	55
Yibing Jia	
1 Purpose	55
2 Theory	56
3 Equipment	61
4 Materials	61
5 Prepare Workspace	62
6 Protocol	62
CHAPTER 4 Single-Cell Gel Electrophoresis Combined with Lesion-Specific Enzymes to Measure Oxidative Damage to DNA	69
Andrew R. Collins, Amaya Azqueta	
1 Purpose	70
2 Theory	70
3 Equipments	72
4 Materials	73
5 Protocol	76

CHAPTER 5	High-Throughput Methods for the Identification of Protein Purification Conditions Using a Cleavable Tag System	93
	Mads Gabrielsen, Katherine S. H. Beckham, Andrew J. Roe	
1	Purpose	94
2	Theory	94
3	Equipment	95
4	Materials.....	95
5	Protocol	98
CHAPTER 6	Two-Dimensional Difference Gel Electrophoresis (2D DIGE)...	111
	Jonathan S. Minden	
1	Purpose.....	112
2	Theory	112
3	Equipment	115
4	Materials.....	116
5	Protocol	121
CHAPTER 7	Optimization of Cell Cycle Measurement by Time-Lapse Microscopy	143
	Gabor Nagy, Gabor Kiraly, Gaspar Banfalvi	
1	Purpose.....	144
2	Theory	144
3	Determination of Cell Cycle Length.....	146
4	Equipments.....	148
5	Materials.....	149
6	Protocol	150
CHAPTER 8	Transfection Methods	163
	A. Ruedel, A. K. Bosserhoff	
1	Purpose.....	164
2	Theory	164
3	Equipment	165
4	Materials.....	165
5	Protocol	166
CHAPTER 9	Cell Enrichment from Human Blood through Red Cell Lysis....	183
	Brandon L. Miller, Jeffrey J. Chalmers, William G. Lowrie	
1	Purpose.....	183
2	Theory	184
3	Equipments.....	185
4	Materials.....	185
5	Protocol	186

CHAPTER 10	Magnetic, Batch Separation	193
	Jeffrey J. Chalmers, William G. Lowrie	
1	Purpose.....	193
2	Theory	194
3	Equipment	195
4	Materials.....	195
5	Protocol	196
CHAPTER 11	Lateral Flow Sandwich Assay Utilizing Upconverting Phosphor (UCP) Reporters	203
	Elisa M. Tjon Kon Fat, William R. Abrams, R. Sam Niedbala, Paul L. A. M. Corstjens	
1	Purpose.....	204
2	Theory	204
3	Equipments.....	208
4	Materials.....	209
5	Protocol	212
CHAPTER 12	In Vivo Modulation of MicroRNA Levels during Spinal Cord Regeneration	235
	Juan Felipe Diaz Quiroz, Karen Echeverri	
1	Purpose.....	236
2	Theory	236
3	Equipments.....	236
4	Materials.....	237
5	Protocol	237
CHAPTER 13	CometChip: Single-Cell Microarray for High-Throughput Detection of DNA Damage	247
	Jing Ge, David K. Wood, David M. Weingeist, Sangeeta N. Bhatia, Bevin P. Engelward	
1	Purpose.....	248
2	Theory	248
3	Equipments.....	249
4	Materials.....	249
5	Protocol	252
CHAPTER 14	Purification of Cardiac Cells	269
	Fumiyuki Hattori, Keiichi Fukuda	
1	Theory	270
2	Equipments.....	270
3	Materials.....	270
4	Protocol	272

CHAPTER 15	Tube Formation <i>In Vitro</i> Angiogenesis Assay.....	281
	Diego Guidolin, Giovanna Albertin	
1	Purpose.....	282
2	Theory.....	282
3	Equipments.....	283
4	Materials.....	284
5	Protocol.....	284
CHAPTER 16	Processing of Transmission Electron Microscopy Images for Single-Particle Analysis of Macromolecular Complexes.....	295
	Zaëlle Devaux, Carlos Oscar S. Sorzano, Slavica Jonic	
1	Purpose.....	296
2	Theory.....	296
3	Equipment.....	299
4	Materials.....	299
5	Protocol.....	301
CHAPTER 17	An Asymmetric 3D <i>In Vitro</i> Assay for the Study of Tumor Cell Invasion.....	311
	Vera Brekhman, Gera Neufeld	
1	Purpose.....	312
2	Theory.....	312
3	Equipment.....	314
4	Materials.....	315
5	Protocol.....	316
CHAPTER 18	Isolation of Myoblasts from Muscle Tissue by Preplating.....	329
	Yoon Ghil Park, Ahmi Baek, Sunmi Kim	
1	Purpose.....	330
2	Theory.....	330
3	Equipment.....	330
4	Materials.....	330
5	Protocol.....	331
CHAPTER 19	High-Content Small Molecule Screening Strategies for Stem-Cell-Derived Motor Neurons.....	339
	Eunju Pack-Chung, Courtney A. Aceffi, Lida Katsimpardi, Kevin J. Kim, Maureen Sherry-Lynes, Nina R. Makhortova, Lee L. Rubin	
1	Theory.....	340
2	Equipment.....	341
3	Materials.....	342
4	Protocol.....	345

CHAPTER 20	A Simple Technique for Quantifying Apoptosis in 96-Well Plates.....	361
	Shilo M. Smith, Deborah Ribble, Nathaniel B. Goldstein, David A. Norris, and Yiqun G. Shellman	
1	Purpose.....	361
2	Theory.....	362
3	Equipments.....	362
4	Materials.....	363
5	Protocol.....	364
CHAPTER 21	Cell Screening Using Resistive-Pulse Sensing.....	369
	Karthik Balakrishnan, Lydia L. Sohn	
1	Purpose.....	370
2	Theory.....	370
3	Equipment.....	372
4	Materials.....	373
5	Protocol.....	373
CHAPTER 22	Quantitative Analysis of Basic Motile Behavior in Amoeboid Cells.....	389
	Deborah Wessels, Spencer Kuhl, and David R. Soll	
1	Purpose.....	390
2	Theory.....	390
3	Equipment.....	390
4	Materials.....	391
5	Protocol.....	392
CHAPTER 23	Single Cell Deposition.....	403
	Jun Liu, Christopher Moraes, Zhe Lu, Craig Simmons, Yu Sun	
1	Purpose.....	404
2	Theory.....	404
3	Equipments.....	406
4	Materials.....	407
5	Protocol.....	408
CHAPTER 24	Enzyme Sensors for Living Cells.....	421
	Ning Chen, Shen Tang, Shenghui Xue, Jenny J. Yang	
1	Purpose.....	422
2	Theory.....	422
3	Equipment.....	422
4	Materials.....	423
5	Protocol.....	425

CHAPTER 25 Myoblast Purifying by Magnetic-Activated Cell Sorting (MACS)	439
Yoon Ghil Park, Ahmi Baek, Sunmi Kim	
1 Purpose	440
2 Theory	440
3 Equipment	440
4 Materials	440
5 Protocol	441
Subject Index	449
Volumes in Series	465