

Contents

Contributors.....	xiii
Preface	xvii
CHAPTER 1 Methods for Monitoring Lysosomal Morphology.....	1
Bethan S. Kilpatrick, Emily R. Eden, Leanne N. Hockey,	
Clare E. Futter, Sandip Patel	
Introduction.....	2
1. Lysosomal Form	2
2. Lysosomal Function.....	3
3. Lysosomal Failure.....	5
4. Linking Lysosomal Form and Failure	6
5. Methods	6
6. Discussion	15
Acknowledgments	16
References	16
CHAPTER 2 A Rapid Method for the Preparation of Ultrapure,	
Functional Lysosomes Using Functionalized	
Superparamagnetic Iron Oxide Nanoparticles.....	21
Mathew W. Walker, Emyr Lloyd-Evans	
1. The Endocytic System	22
2. The Discovery of Lysosomes and Lysosomal Storage Diseases	23
3. Biochemical Features of Lysosomes.....	24
4. Overview of Methods for Purifying Lysosomes.....	24
5. Method for Magnetic Separation of Lysosomes from	
Whole Cells	28
6. Technical Considerations.....	31
7. Techniques for Determining Purity of the Lysosomal Fractions	38
8. Discussion	40
Acknowledgments	41
References	41
CHAPTER 3 TFEB and the CLEAR Network.....	45
Carmine Settembre, Diego L. Medina	
Introduction.....	46
1. TFEB Nuclear Translocation Assay.....	47
2. Cell Culture and Treatment.....	48
References	61

CHAPTER 4	Biosynthesis, Targeting, and Processing of Lysosomal Proteins: Pulse–Chase Labeling and Immune Precipitation	63
	Sandra Pohl, Andrej Hasilik	
	Introduction.....	64
	Briefly on Functions and Biogenesis of Lysosomes	64
	On Application of Radioactive Isotopes in Studies of Molecular Forms of Lysosomal Proteins.....	67
	1. Materials	69
	2. Methods	71
	3. Metabolic Labeling and Immune Precipitation of the Lysosomal Protease Cathepsin Z.....	77
	4. Discussion	80
	Acknowledgments	82
	References	82
CHAPTER 5	Measuring Lysosomal pH by Fluorescence Microscopy.....	85
	Johnathan Canton, Sergio Grinstein	
	Introduction.....	86
	Instrumentation.....	89
	Probe Selection.....	90
	Calibration.....	92
	1. Materials	93
	2. Methods	94
	Conclusion.....	95
	Notes	96
	Acknowledgments	96
	References	96
CHAPTER 6	Lysosome Fusion in Cultured Mammalian Cells	101
	Nicholas A. Bright, Lena Wartosch, J. Paul Luzio	
	1. Overview of Methods to Study Lysosome Fusion.....	102
	2. Choosing an Assay System	106
	3. Methods	109
	Acknowledgments	116
	References	116
CHAPTER 7	RNAi Screens of Lysosomal Trafficking	119
	Salil Garg, Michael B. Brenner	
	1. Methods of Gene Depletion in Lysosomes.....	120

2. Selection of Screening System.....	120
3. Assay Validation.....	126
4. Validation of Hits.....	133
5. Discussion.....	135
Acknowledgments.....	136
References.....	136
CHAPTER 8 Approaches for Plasma Membrane Wounding and Assessment of Lysosome-Mediated Repair Responses.....	139
M. Corrotte, T. Castro-Gomes, A.B. Koushik, N.W. Andrews	
1. Overview of Wounding Methods and Plasma Membrane Repair Mechanisms.....	140
2. Procedures for Plasma Membrane Wounding.....	142
3. Procedures for Measuring the Extent of Plasma Membrane Repair.....	149
4. Procedures to Measure Exocytosis of Lysosomes.....	152
Acknowledgments.....	157
References.....	157
CHAPTER 9 Imaging Approaches to Measuring Lysosomal Calcium.....	159
Anthony J. Morgan, Lianne C. Davis, Antony Galione	
1. Endolysosomal Ca^{2+}	160
2. Assessing Organelle Ca^{2+} : General Strategies.....	161
3. Assessing Endolysosomal Ca^{2+} : Specific Strategies.....	168
4. Final Remarks.....	189
References.....	189
CHAPTER 10 Lysosome Electrophysiology.....	197
Xi Z. Zhong, Xian-Ping Dong	
Introduction.....	198
1. Lysosome.....	198
2. Materials.....	203
3. Methods.....	204
4. Discussion.....	210
5. Summary.....	211
Acknowledgments.....	211
References.....	211

CHAPTER 11	Reconstitution of Lysosomal Ion Channels into Artificial Membranes.....	217
	Elisa Venturi, Rebecca Sitsapesan	
	Introduction.....	218
1.	The Bilayer Apparatus	218
2.	Electrical Equipment Used for Single-Channel Recordings..	220
3.	Painting Bilayers.....	222
4.	Ion Channel Incorporation into a Bilayer.....	223
5.	Single-Channel Current Amplitude and Conductance Measurements.....	225
6.	Choice of Permeant Ion.....	227
7.	Measuring the Relative Permeability of Different Ions.....	229
8.	Measurements of Liquid Junction Potentials	230
9.	Single-Channel Gating and Measurements of Open Probability	230
10.	Noise Analysis.....	231
11.	Isolation of Native and Recombinant Purified Lysosomal Ion Channels	232
12.	Discussion.....	234
	References	234
CHAPTER 12	Fluorescence Methods for Analysis of Interactions between Ca^{2+} Signaling, Lysosomes, and Endoplasmic Reticulum.....	237
	David L. Prole, Cristina I. López-Sanjurjo, Stephen C. Tovey, Colin W. Taylor	
1.	ER, Lysosomes, and Ca^{2+} Signaling.....	238
2.	Pharmacological Tools	240
3.	Fluorescence Methods.....	241
4.	Fluorescence Tools for Analysis of Lysosomes.....	243
5.	Ca^{2+} Signaling and Lysosomes: Tools and Practical Problems	245
6.	Single-cell Analyses of Cytosolic Ca^{2+} Signals.....	247
7.	High-throughput Analyses of Cytosolic Ca^{2+} Signals.....	250
8.	Tracking Interactions between Lysosomes and ER by Fluorescence Microscopy	251
	Conclusions.....	254
	Acknowledgments	254
	References	255

CHAPTER 13 Methods for the Quantification of Lysosomal Membrane Permeabilization: A Hallmark of Lysosomal Cell Death	261
Sonja Aits, Marja Jäätelä, Jesper Nylandsted	
Introduction	263
Method 1: Quantification of Cathepsin and β -N-acetylglucosaminidase Release into the Cytosol by Enzymatic Activity Measurement	266
1. Materials	266
2. Protocol	267
Method 2: LMP Visualized by Release of Fluorescent Dextran to the Cytosol	271
3. Materials	273
4. Protocol	273
Method 3: LMP Visualized by Cathepsin Immunocytochemistry	274
5. Materials	275
6. Protocol	275
Method 4: Detection of Damaged Lysosomes by Galectin-1 and -3 Translocation	278
7. Materials	278
8. Protocol	279
Discussion	281
Acknowledgments	283
References	283
CHAPTER 14 Measuring the Phagocytic Activity of Cells	287
Nick Platt, Paul Fineran	
Introduction	288
1. Reasons to Undertake Studies of Phagocytosis	289
2. Components of a Phagocytosis Assay	289
3. In vitro or In vivo Study?	289
4. Methodologies for Analyses of Phagocytosis	290
5. Selection of Phagocyte Population	291
6. Choice of Target Particle	293
7. Targeting Particles to Specific Phagocytic Receptors	294
8. Detection of Ingested Particles	294
9. Protocol 1. Fc γ Receptor-Mediated Phagocytosis of IgG Opsonized Sheep Red Blood Cells by Murine Macrophages	295

10. Materials and Reagents	295
11. Equipment	296
12. Protocol	296
13. Protocol 2. FACS Analysis of <i>Mycobacterium bovis</i> Internalization by RAW264.7 Cells	298
14. Materials	299
15. Equipment	300
16. Summary	302
Acknowledgments	302
References	302
CHAPTER 15 Detection and Quantification of Microbial Manipulation of Phagosomal Function.....	305
Maria Podinovskaia, David G. Russell	
Introduction.....	306
An Overview of the Role of the Macrophage Phagosome in Infection.....	306
The Role of Lysosomes in Microbial Killing.....	307
Methods Utilized in the Study of Lysosome Function.....	308
1. Considerations for Choice of Reagents, Cells, and Readouts..	309
2. Reagents.....	312
3. Methods	316
4. Analytical Platforms, Data Collection and Analysis	320
5. Discussion	326
6. Summary.....	327
Acknowledgments	327
Supplementary Data.....	328
References	328
CHAPTER 16 Measuring Relative Lysosomal Volume for Monitoring Lysosomal Storage Diseases	331
Danielle te Vrugte, Kerri L. Wallom, Frances M. Platt	
1. Measuring Relative Lysosomal Volume as an Index of Lysosomal Storage	332
2. In Which Circulating Cell Type Should Relative Lysosomal Volume Be Measured?	333
3. How to Measure Relative Lysosomal Volume in Blood Cells.	334
4. Methods	336
5. Summary.....	346
Acknowledgments	346
References	347

CHAPTER 17 Quantifying Storage Material Accumulation in Tissue Sections	349
Jonathan D. Cooper, Helen R.E. Brooks, Hemanth R. Nelvagal	
Introduction	350
1. Detection of Storage Material	350
2. Quantification of Storage Material	354
Conclusion	355
References	355
CHAPTER 18 Laboratory Diagnosis of Niemann–Pick Disease Type C: The Filipin Staining Test	357
Marie T. Vanier, Philippe Latour	
Introduction and Rationale	358
1. Materials	360
2. Methods	361
3. Discussion	367
Concluding Remarks	373
Acknowledgments	373
References	373
Volumes in Series	377
Index	389