

*Methods in Enzymology*

*Volume 298*

*Molecular Motors and  
the Cytoskeleton  
Part B*

EDITED BY

*Richard B. Vallee*

WORCESTER FOUNDATION FOR BIOMEDICAL RESEARCH  
SHREWSBURY, MASSACHUSETTS



ACADEMIC PRESS

San Diego London Boston New York Sydney Tokyo Toronto

## Table of Contents

CONTRIBUTORS TO VOLUME 298. . . . .	ix
PREFACE. . . . .	xiii
VOLUMES IN SERIES . . . . .	xv

### Section I. Analysis of Actomyosin-Related Systems

1. Purification and Assay of Myosin V	RICHARD E. CHENEY	3
2. Use of Latrunculin-A, an Actin Monomer-Binding Drug	KATHRYN AYS COUGH	18
3. Use of the F-Actin Binding Drugs, Misakinolide A and Swinholide A	MICHAEL R. BUBB AND ILAN SPECTOR	26
4. F-Actin Blot Overlays	ELIZABETH J. LUNA	32
5. Purification and Assay of the Arp2/3 Complex from <i>Acanthamoeba castellanii</i>	JOSEPH F. KELLEHER, R. DYCHE MULLINS, AND THOMAS D. POLLARD	42
6. Purification and Assay of the Platelet Arp2/3 Complex	MATTHEW D. WELCH AND TIMOTHY J. MITCHISON	52
7. Purification and Assay of Zyxin	KAREN L. SCHMEICHEL, BETH E. STRONACH, AND MARY C. BECKERLE	62
8. Purification and Assays for Paxillin	CHRISTOPHER E. TURNER AND MICHAEL C. BROWN	77
9. Assay and Purification of Focal Adhesion Kinase	JUDITH LACOSTE, AMY MA, AND J. THOMAS PARSONS	89
10. Purification and Assays of VASP	THOMAS JARCHAU, THOMAS MUND, MATTHIAS REINHARD, AND ULRICH WALTER	103
11. <i>Listeria monocytogenes</i> -Based Assays for Actin Assembly Factors	JULIE A. THERIOT AND DAVID C. FUNG	114

---

## Section II. Analysis of Microtubule-Related Systems

12. Preparation and Characterization of Caged Fluorescein Tubulin	ARSHAD DESAI AND TIMOTHY J. MITCHISON	125
13. Purification of Novel Kinesins from Embryonic Systems	DAVID MEYER, DANIEL R. RINES, ANNA KASHINA, AND JONATHAN M. SCHOLEY	133
14. Assaying Processive Movement of Kinesin by Fluorescence Microscopy	DANIEL W. PIERCE AND RONALD D. VALE	154
15. Purification of Dynactin and Dynein from Brain Tissue	JAMES B. BINGHAM, STEPHEN J. KING, AND TRINA A. SCHROER	171
16. Isolation and Characterization of Kinectin	JANARDAN KUMAR AND MICHAEL P. SHEETZ	185
17. Purification and Assay of CLIP-170	GEORGIOS S. DIAMANTOPOULOS, THOMAS E. KREIS, AND JANET E. RICKARD	197
18. Purification and Assay of the Microtubule-Severing Protein, Katanin	FRANK MCNALLY	206
19. Purification and Assay of $\gamma$ Tubulin Ring Complex	YIXIAN ZHENG, MEI LIE WONG, BRUCE ALBERTS, AND TIMOTHY J. MITCHISON	218
20. Rapid Isolation of Centrosomes	MAUREEN BLOMBERG-WIRSHELL AND STEPHEN J. DOXSEY	228
21. Photoaffinity Labeling Approach to Map Taxol-Binding Site on the Microtubule	GEORGE A. ORR, SRINIVASA RAO, CHARLES S. SWINDELL, DAVID G. I. KINGSTON, AND SUSAN BAND HORWITZ	238
22. Use of Drugs to Study Role of Microtubule Assembly Dynamics in Living Cells	MARY ANN JORDAN AND LESLIE WILSON	252

## Section III. Other Cytoskeletal Systems

23. Purification and Assay of a Septin Complex from <i>Drosophila</i> Embryos	KAREN OEGEMA, ARSHAD DESAI, MEI LIE WONG, TIMOTHY J. MITCHISON, AND CHRISTINE M. FIELD	279
---	--	-----

24. Purification, Assembly, and Localization of FtsZ	AMIT MUKHERJEE AND JOE LUTKENHAUS	296
25. Purification and Assembly of FtsZ	CHUNLIN LU AND HAROLD P. ERICKSON	305

#### Section IV. Cell-Free and Genetic Systems

26. High-Resolution Video and Digital Enhanced Differential Interference Contrast Light Microscopy of Cell Division in Budding Yeast	E. D. SALMON, ELAINE YEH, SID SHAW, BOB SKIBBENS, AND KERRY BLOOM	317
27. Production of M-Phase and I-Phase Extracts from Mammalian Cells	DUANE A. COMPTON	331
28. Organelle Motility and Membrane Network Formation in Metaphase and Interphase Cell-Free Extracts	VIKI J. ALLAN	339
29. Organelle Motility and Membrane Network Formation from Cultured Mammalian Cells	CAROL MCGOLDRICK AND MICHAEL P. SHEETZ	353
30. An <i>in Vitro</i> Motility Assay for Melanophore Pigment Organelles	STEPHEN L. ROGERS, IRINA S. TINT, AND VLADIMIR I. GELFAND	361
31. Purification of Phagosomes and Assays for Microtubule Binding	JANIS K. BURKHARDT	373
32. Magnetic Bead Assay for Characterization of Microtubule-Membrane Interactions	JOCHEN SCHEEL AND THOMAS E. KREIS	381
33. Reactivation of Vesicle Transport in Lysed Teleost Melanophores	LEAH HAIMO	389
34. Reactivable Cell Models of Giant Amoeba <i>Reticulomyxa</i>	MICHAEL P. KOONCE, URSULA EUTENEUER, AND MANFRED SCHLIWA	400

#### Section V. Force Production Assays

35. Design and Use of the Centrifuge Microscope to Assay Force Production	RONALD J. BASKIN	413
36. Motility Assays on Molluscan Native Thick Filaments	YUNG JIN HAN AND JAMES R. SELLERS	427
37. Use of Optical Traps in Single Molecule Study on Nonprocessive Biological Motors	AMIT D. MEHTA, JEFFREY T. FINER, AND JAMES A. SPUDICH	436
38. Versatile Optical Traps with Feedback Control	KOEN VISSCHER AND STEVEN M. BLOCK	460

39. Preparation of a Flexible, Porous Polyacrylamide Substrate for Mechanical Studies of Cultured Cells	YU-LI WANG AND ROBERT J. PELHAM, JR.	489
40. Design and Use of Substrata to Measure Traction Forces Exerted by Cultured Cells	TIM OLIVER, KEN JACOBSON, AND MICAH DEMBO	497

### Section VI. General Methods

41. Purification of Cytoskeletal Proteins Using Peptide Antibodies	CHRISTINE M. FIELD, KAREN OEGEMA, YIXIAN ZHENG, TIMOTHY J. MITCHISON, AND CLAIRE E. WALCZAK	525
42. Strategies to Assess Phosphoprotein Phosphatase and Protein Kinase-Mediated Regulation of the Cytoskeleton	JOHN E. ERIKSSON, DIANA M. TOIVOLA, CECILIA SAHLGREN, ANDREY MIKHAILOV, AND ANN-SOFI HÄRMÄLÄ-BRASKÉN	542
43. Correlative Light and Electron Microscopy of the Cytoskeleton of Cultured Cells	TATYANA M. SVITKINA AND GARY G. BORISY	570
AUTHOR INDEX . . . . .		593
SUBJECT INDEX . . . . .		619