This is a free sample of content from Subcellular Fractionation. Click here for more information on how to buy the book.

SUBCELLULAR FRACTIONATION

A LABORATORY MANUAL

ALSO FROM COLD SPRING HARBOR LABORATORY PRESS

RELATED TITLES

Antibodies: A Laboratory Manual, Second Edition

Calcium Techniques: A Laboratory Manual

Cell Survival and Cell Death

Endocytosis

Endoplasmic Reticulum

The Golgi

Mitochondria

Origin and Evolution of Eukaryotes

Signal Transduction: Principles, Pathways, and Processes

LABORATORY MANUALS

Imaging: A Laboratory Manual

Imaging in Developmental Biology: A Laboratory Manual

Imaging in Neuroscience: A Laboratory Manual

Live Cell Imaging: A Laboratory Manual, Second Edition

Manipulating the Mouse Embryo: A Laboratory Manual, Fourth Edition

Molecular Cloning: A Laboratory Manual, Fourth Edition

Molecular Neuroscience: A Laboratory Manual Mouse Models of Cancer: A Laboratory Manual

Purifying and Culturing Neural Cells: A Laboratory Manual

RNA: A Laboratory Manual

HANDBOOKS

At the Bench: A Laboratory Navigator, Updated Edition At the Helm: Leading Your Laboratory, Second Edition Experimental Design for Biologists, Second Edition

Lab Math: A Handbook of Measurements, Calculations, and Other Quantitative Skills for Use at the Bench

Lab Ref: A Handbook of Recipes, Reagents, and Other Reference Tools for Use at the Bench, Volume 1 and Volume 2

Statistics at the Bench: A Step-by-Step Handbook for Biologists

WEBSITE

www.cshprotocols.org

SUBCELLULAR FRACTIONATION

A LABORATORY MANUAL

EDITED BY

Paul R. Pryor

Centre for Immunology and Infection Hull York Medical School and Department of Biology University of York



SUBCELLULAR FRACTIONATION

A LABORATORY MANUAL

All rights reserved

© 2015 Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Printed in the United States of America

Publisher John Inglis
Acquisition Editor Judy Cuddihy
Director of Editorial Development Jan Argentine
Managing Editor Maria Smit
Developmental Editor Kaaren Janssen
Project Manager Maryliz Dickerson

Production Editors Kathleen Bubbeo and Joanne McFadden

Production Manager Denise Weiss
Director of Product Development & Marketing Wayne Manos
Cover Designer Michael Albano

Cover art: Outline of steps involved in the isolation of specific organelles from a crude cell homogenate using a step gradient. Many protocols in this manual employ this and other differential centrifugation strategies to fractionate cells.

Library of Congress Cataloging-in-Publication Data

Subcellular fractionation: a laboratory manual / edited by Paul R. Pryor.

p.; cm

Includes bibliographical references and index.

ISBN 978-1-62182-038-3 (cloth) -- ISBN 978-1-62182-042-0 (pbk.)

I. Pryor, Paul R., editor.

[DNLM: 1. Subcellular Fractions--Laboratory Manuals. 2. Cell Fractionation--methods--Laboratory Manuals.

3. Cellular Structures--Laboratory Manuals. QU 25]

QH581.2 611'.0181-dc23

2014034658

10 9 8 7 6 5 4 3 2 1

Students and researchers using the procedures in this manual do so at their own risk. Cold Spring Harbor Laboratory makes no representations or warranties with respect to the material set forth in this manual and has no liability in connection with the use of these materials. All registered trademarks, trade names, and brand names mentioned in this book are the property of the respective owners. Readers should please consult individual manufacturers and other resources for current and specific product information.

With the exception of those suppliers listed in the text with their addresses, all suppliers mentioned in this manual can be found on the BioSupplyNet website at www.biosupplynet.com.

All World Wide Web addresses are accurate to the best of our knowledge at the time of printing.

Procedures for the humane treatment of animals must be observed at all times. Check with the local animal facility for guidelines.

Certain experimental procedures in this manual may be the subject of national or local legislation or agency restrictions. Users of this manual are responsible for obtaining the relevant permissions, certificates, or licenses in these cases. Neither the authors of this manual nor Cold Spring Harbor Laboratory assume any responsibility for failure of a user to do so.

The materials and methods in this manual may infringe the patent and proprietary rights of other individuals, companies, or organizations. Users of this manual are responsible for obtaining any licenses necessary to use such materials and to practice such methods. COLD SPRING HARBOR LABORATORY MAKES NO WARRANTY OR REPRESENTATION THAT USE OF THE INFORMATION IN THIS MANUAL WILL NOT INFRINGE ANY PATENT OR OTHER PROPRIETARY RIGHT.

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Cold Spring Harbor Laboratory Press, provided that the appropriate fee is paid directly to the Copyright Clearance Center (CCC). Write or call CCC at 222 Rosewood Drive, Danvers, MA 01923 (978-750-8400) for information about fees and regulations. Prior to photocopying items for educational classroom use, contact CCC at the above address. Additional information on CCC can be obtained at CCC Online at www.copyright.com.

For a complete catalog of all Cold Spring Harbor Laboratory Press publications, visit our website at www.cshlpress.org.

Contents

	Acknowledgments	x
CHAPTER 1	Introduction	
	Introduction Paul R. Pryor	1
CHAPTER 2	Lectin-Magnetic Beads for Plasma Membrane Isolation	
	PROTOCOL	
	Lectin-Magnetic Beads for Plasma Membrane Isolation Yu-Chen Lee, Hsuan-Chen Liu, Carol Chuang, and Sue-Hwa Lin	ŗ
CHAPTER 3	Isolation of Clathrin-Coated Vesicles from Tissue Culture Cells	
	INTRODUCTION	
	Isolation of Clathrin-Coated Vesicles from Tissue Culture Cells Georg H.H. Borner and Andrew B. Fielding	11
	PROTOCOLS	
	1 Isolating HeLa Cell Fractions Enriched for Clathrin-Coated Vesicles Georg H.H. Borner and Andrew B. Fielding	14
	2 Using In-Gel Digestion and an Orbitrap Mass Spectrometer to Analyze the Proteome of Clathrin-Coated Vesicles Georg H.H. Borner and Andrew B. Fielding	18
	3 Using In-Solution Digestion, Peptide Fractionation, and a Q Exactive Mass Spectrometer to Analyze the Proteome of Clathrin-Coated Vesicles Georg H.H. Borner and Andrew B. Fielding	22
CHAPTER 4	Purification of Early and Late Endosomes	
	- Turneation of Early and Eace Endosomes	
	INTRODUCTION	
	Purification of Early and Late Endosomes Mariana E.G. Araújo, Giorgia Lamberti, and Lukas A. Huber	27
	PROTOCOLS	
	1 Homogenization of Mammalian Cells Mariana E.G. Araújo, Giorgia Lamberti, and Lukas A. Huber	32
		`

vi /	Contents
------	----------

	2 Isolation of Early and Late Endosomes by Density Gradient Centrifugation Mariana E.G. Araújo, Giorgia Lamberti, and Lukas A. Huber	36
	3 Isolation of Macrophage Early and Late Endosomes by Latex Bead Internalization and Density Gradient Centrifugation	40
	Giorgia Lamberti, Mariana E.G. Araújo, and Lukas A. Huber	
CHAPTER 5	The Synaptosome as a Model System for Studying Synaptic Physiology	
	INTRODUCTION	
	The Synaptosome as a Model System for Studying Synaptic Physiology Gareth J.O. Evans	45
	PROTOCOL	
	1 Subcellular Fractionation of the Brain: Preparation of Synaptosomes and Synaptic Vesicles Gareth J.O. Evans	49
CHAPTER 6	Isolating Phagosomes from Tissue Culture Cells	
	PROTOCOL	
	Isolating Phagosomes from Tissue Culture Cells Paul R. Pryor and Adam P. Rofe	55
CHAPTER 7	Strategies for Isolation of Exosomes	
	INTRODUCTION	
	Strategies for Isolation of Exosomes Emily Zeringer, Timothy Barta, Mu Li, and Alexander V. Vlassov	59
CHAPTER 8	Isolation of Lysosomes from Rat Tissue and Tissue Culture Cells	
	INTRODUCTION	
	Isolation of Lysosomes from Rat Tissue and Tissue Culture Cells Paul R. Pryor	65
	PROTOCOLS	
	1 Isolating Lysosomes from Rat Liver Paul R. Pryor	67
	2 Purification of Lysosomes Using Supraparamagnetic Iron Oxide Nanoparticles (SPIONs) Adam P. Rofe and Paul R. Pryor	72

CHAPTER 9	Isolation of Specialized Secretory Compartments: GLU14 Storage Vesicles		
	INTRODUCTION		
	Isolation of Specialized Secretory Compartments: GLUT4 Storage Vesicles Jessica B.A. Sadler, Christopher A. Lamb, Gwyn W. Gould, and Nia J. Bryant	77	
	PROTOCOLS		
	1 Preparation of a Total Membrane Fraction from 3T3-L1 Adipocytes Jessica B.A. Sadler, Christopher A. Lamb, Gwyn W. Gould, and Nia J. Bryant	79	
	2 16K Fractionation of 3T3-L1 Adipocytes to Produce a Crude GLUT4-Containing Vesicle Fraction Jessica B.A. Sadler, Christopher A. Lamb, Gwyn W. Gould, and Nia J. Bryant	82	
	3 Complete Membrane Fractionation of 3T3-L1 Adipocytes Jessica B.A. Sadler, Christopher A. Lamb, Gwyn W. Gould, and Nia J. Bryant	86	
	4 Iodixanol Gradient Centrifugation to Separate Components of the Low-Density Membrane Fraction from 3T3-L1 Adipocytes Jessica B.A. Sadler, Christopher A. Lamb, Gwyn W. Gould, and Nia J. Bryant	92	
CHAPTER 10	Isolation of Peroxisomes from Yeast		
	INTRODUCTION		
	Isolation of Peroxisomes from Yeast Jana Cramer, Daniel Effelsberg, Wolfgang Girzalsky, and Ralf Erdmann	97	
	PROTOCOLS		
	1 Small-Scale Purification of Peroxisomes for Analytical Applications Jana Cramer, Daniel Effelsberg, Wolfgang Girzalsky, and Ralf Erdmann	100	
	2 Large-Scale Purification of Peroxisomes for Preparative Applications Jana Cramer, Daniel Effelsberg, Wolfgang Girzalsky, and Ralf Erdmann	108	
CHAPTER 11	Isolation and Analysis of Microtubules and Associated Proteins		
	INTRODUCTION		
	Isolation and Analysis of Microtubules and Associated Proteins Roger D. Sloboda	115	
	PROTOCOLS		
	1 Isolation of Microtubules by Assembly/Disassembly Methods Roger D. Sloboda	118	
	2 Isolation of Microtubules and Microtubule-Associated Proteins Using Paclitaxel Roger D. Sloboda	123	
	3 Separation of Tubulin and Microtubule-Associated Proteins by Ion Exchange Chromatography *Roger D. Sloboda**	127	

viii	/	Contents
VIII	/	Contents

	4 Isolation of Microtubule-Based Motor Proteins by ATP Release from Paxlitaxel-Stabilized Microtubules	131
	Roger D. Sloboda	120
	5 Observation of Microtubule-Based Motor Protein Activity Roger D. Sloboda	136
CHAPTER 12	Golgi Isolation	
	PROTOCOL	
	Golgi Isolation Danming Tang and Yanzhuang Wang	141
CHAPTER 13	Isolation of Mitochondria from Cells and Tissues	
	INTRODUCTION	
	Isolation of Mitochondria from Cells and Tissues David A. Clayton and Gerald S. Shadel	147
	PROTOCOLS	
	1 Isolation of Mitochondria from Tissue Culture Cells David A. Clayton and Gerald S. Shadel	149
	2 Isolation of Mitochondria from Animal Tissue David A. Clayton and Gerald S. Shadel	152
	3 Purification of Mitochondria by Sucrose Step Density Gradient Centrifugation David A. Clayton and Gerald S. Shadel	155
CHAPTER 14	Isolation of Chloroplasts from Plant Protoplasts	
	PROTOCOL	
	1 Isolation of Chloroplasts from Plant Protoplasts Shiu-Cheung Lung, Matthew D. Smith, and Simon D.X. Chuong	159
CHAPTER 15	Subcellular Fractionation of Rough Microsomes	
	INTRODUCTION	
	Subcellular Fractionation of Rough Microsomes David D. Sabatini	165
	PROTOCOLS	
	1 Preparation of Rough Microsomes from Rat Liver David D. Sabatini	168

Contents / ix

	2 Preparation of Crude Rough Microsomes from Dog Pancreas David D. Sabatini	175
	3 Preparation of Crude Rough Microsomes from Tissue Culture Cells David D. Sabatini	179
CHAPTER 16	Purification of Ribosomes, Ribosomal Subunits, and Polysomes	
	INTRODUCTION	
	Purification of Ribosomes, Ribosomal Subunits, and Polysomes Maria C. Rivera, Bruce Maguire, and James A. Lake	187
	PROTOCOLS	
	1 Isolation of Ribosomes and Polysomes Maria C. Rivera, Bruce Maguire, and James A. Lake	189
	2 Isolation of Ribosomes by Chromatography Bruce A. Maguire	196
	3 Purification of 70S Ribosomes Maria C. Rivera, Bruce Maguire, and James A. Lake	200
	4 Purification of Polysomes Maria C. Rivera, Bruce Maguire, and James A. Lake	203
	5 Dissociation of Ribosomes into Large and Small Subunits Maria C. Rivera, Bruce Maguire, and James A. Lake	206
CHAPTER 17	Isolation of Nuclei	
	INTRODUCTION	
	Isolation of Nuclei Arash Nabbi and Karl Riabowol	211
	PROTOCOLS	
	1 Rapid Isolation of Nuclei from Cells In Vitro Arash Nabbi and Karl Riabowol	215
	2 Isolation of Pure Nuclei Using a Sucrose Cushion Arash Nabbi and Karl Riabowol	219
CHAPTER 18	The Nuclear Matrix: Fractionation Techniques and Analysis	
	INTRODUCTION	
	The Nuclear Matrix: Fractionation Techniques and Analysis Rosemary H.C. Wilson, Emma L. Hesketh, and Dawn Coverley	223

X	/ Contents

	PROTOCOL	
	1 Preparation of the Nuclear Matrix for Parallel Microscopy and Biochemical Analyses Rosemary H.C. Wilson, Emma L. Hesketh, and Dawn Coverley	228
APPENDIX	General Safety and Hazardous Material Information	
	General Safety and Hazardous Material Information	235
	Index	241

Acknowledgments

I would like to acknowledge all the authors and especially the staff at CSHLP—Judy Cuddihy, Maryliz Dickerson, and Maria Smit—for their patience in bringing this book to fruition.

This is a free sample of content from Subcellular Fractionation. Click here for more information on how to buy the book.

This manual should be used by laboratory personnel with experience in laboratory and chemical safety or students under the supervision of such trained personnel. The procedures, chemicals, and equipment referenced in this manual are hazardous and can cause serious injury unless performed, handled, and used with care and in a manner consistent with safe laboratory practices. Students and researchers using the procedures in this manual do so at their own risk. It is essential for your safety that you consult the appropriate Material Safety Data Sheets, the manufacturers' manuals accompanying equipment, and your institution's Environmental Health and Safety Office, as well as the General Safety and Disposal Cautions in the Appendix for proper handling of hazardous materials in this manual. Cold Spring Harbor Laboratory makes no representations or warranties with respect to the material set forth in this manual and has no liability in connection with the use of these materials.

All registered trademarks, trade names, and brand names mentioned in this book are the property of the respective owners. Readers should please consult individual manufacturers and other resources for current and specific product information.

Appropriate sources for obtaining safety information and general guidelines for laboratory safety are provided in the General Safety and Hazardous Material Information appendix of this manual.