

# Index

Page references followed by f denote figures; those followed by t denote tables.

## A

- Acetonitrile, 19–20, 24
- Adaptor protein (AP) complexes, 13
- Adipocytes (3T3-L1) in GLUT4 storage vesicle preparation
  - complete membrane fractionation of 3T3-L1 adipocytes, 86–91
- iodixanol gradient centrifugation to separate components of the low-density membrane fraction from 3T3-L1 adipocytes, 92–94
- preparation of a total membrane fraction from 3T3-L1 adipocytes (protocol), 79–81
- 16K fractionation of 3T3-L1 adipocytes to produce a crude GLUT4-containing vesicle fraction (protocol), 82–85
- Affinity-based capture of exosomes, 61
- Antibodies, in affinity-based capture of exosomes, 61
- Arabidopsis thaliana*, chloroplast isolation from protoplasts of, 159–163
- Axonemes, 139–140

## B

- Ballabio, Andreas, 65
- BCA protein assay, 16
- $\beta$ -hexosaminidase activity, 69
- Binding medium for phagosomes (recipe), 57
- Bovine brain tissue, collecting, 119
- Brain
  - isolation of microtubules by assembly/disassembly methods (protocol), 118–122
  - subcellular fractionation, 49–52, 50f, 51t
  - synaptosomes as model system for studying synaptic physiology, 45–53
- Brain homogenization buffer (recipe), 52
- Buffer A for CCVs (recipe), 16, 21
- Buffer A-FS (recipe), 16

## C

- Cadherins, 9
- Catalase activity buffer (recipe), 105, 113
- Catalase activity measurement, 103, 112, 113
- Centrifugation. *See also* Density gradient centrifugation; *specific protocols*
  - medium for, 3
  - overview, 2–3
  - rotors for, 2–3
- Centrosome, 139
- Chicken brain tissue, collecting, 119
- Chlamydomonas* axonemes, 139–140
- Chloroplast homogenization buffer (recipe), 193
- Chloroplast isolation from plant protoplasts (protocol), 159–163

- discussion, 162
- materials, 159–160
- method, 160–161
  - isolating chloroplasts, 161
  - isolating protoplasts, 160–161
- recipes, 162–163
- Chloroplast lysis buffer (recipe), 193
- Chloroplast ribosome dissociation buffer (recipe), 209
- Chloroplast ribosomes
  - cell extract preparation for, 191, 192
  - density gradient centrifugation, 193
  - dissociation of ribosomes into large and small subunits, 206–210
- Chromatography, isolation of ribosomes by (protocol), 196–199
  - materials, 196–197
  - method, 197–198
  - recipes, 198–199
  - troubleshooting, 198
- Clathrin-coated vesicles (CCVs), isolation from tissue culture cells, 11–25
  - heterogeneity of CCVs within sample, 13
  - in-gel digestion and Orbitrap mass spectrometer to analyze the proteome of CCVs (protocol), 18–21
    - materials, 18–19
    - method, 19–20
      - in-gel digestion, 19
      - mass spectrometry, 20
      - peptide extraction, 20
    - recipe, 21
  - isolating HeLa cell fractions enriched for CCVs (protocol), 14–17
    - materials, 14–15
    - method, 15–16
    - recipes, 16
  - limitations of method, 12–13
  - organ-based methods compared, 11–12
  - proteome analysis of CCVs using in-solution digestion, peptide fractionation, and a Q exactive mass spectrometer (protocol), 22–25
    - materials, 22–23
    - method, 23–25
      - in-solution digestion, 23–24
      - mass spectrometric analysis, 24–25
      - peptide fractionation using SDB-RPS, 24
    - recipes, 25
- Colloidal iron dextran (FeDex) (recipe), 75
- Concanavalin A, in lectin-magnetic beads for plasma membrane isolation (protocol), 5–9, 6f, 8t
- Coomassie Plus protein assay reagent, 8
- Core nuclear matrix, 223, 232–233
- CS-mannitol buffer (recipe), 162
- CS-sucrose buffer (recipe), 162
- Cycloheximide, 180
- Cysteine-SulfoLink resin, 196
- Cytoskeletal (CSK) buffer (recipe), 233

## D

- de Duve, Christian, 65, 97
- Density gradient centrifugation
  - endosome isolation (protocol), 36–39
    - materials, 36
    - method, 37–38
      - continuous gradient, 37–38
      - step gradient, 37
    - recipes, 38
  - iodixanol gradient centrifugation to separate components of the low-density membrane fraction from 3T3-L1 adipocytes (protocol), 92–94
  - isolation of macrophage early and late endosomes by latex bead internalization and (protocol), 40–44
    - discussion, 42, 43f
    - materials, 40–41
    - method, 41–42
      - discontinuous sucrose gradient preparation, 41–42
      - harvesting cells, 41
      - homogenizing cells, 41
      - latex bead internalization, 41
      - separation of organelles containing latex beads, 42
    - recipes, 43–44
    - troubleshooting, 42
  - lysosome isolation from rat liver (protocol), 67–71
    - discussion, 70
    - materials, 67–68
    - method, 68–70
      - density-gradient centrifugation, 69
      - isolation of endolysosomes, 70
      - preparation of homogenate, 68–69
    - recipes, 70–71
  - mitochondria purification by sucrose step density gradient centrifugation (protocol), 155–157
    - materials, 155
    - method, 155–156
    - recipes, 157
    - troubleshooting, 156
- nuclei isolation, 219–222
  - discussion, 221
  - materials, 219–220
  - method, 220–221
  - recipes, 222
- peroxisomes
  - large-scale purification for preparative applications (protocol), 108–114
  - small-scale purification for analytical applications (protocol), 100–107, 104f
- ribosome and polysome isolation, 188, 192–193, 200–210
- rough microsome isolation, 165
- separation of kinensin and dynein by sucrose gradient centrifugation, 134

## Index

- Diethylaminoethyl (DEAE) cellulose, 129  
Diethylaminoethyl (DEAE) Sephadex, 129  
Differential centrifugation, 2–3  
Discontinuous sucrose gradient preparation, 41–42  
Disruption of cells, 1–2. *See also specific methods; specific protocols*  
Dithiobis(succinimidyl propionate), 232  
DNA, nuclear matrix and, 225  
DNase, 223, 225, 228–232  
Dounce homogenizer, 1–2, 151, 152, 170, 176–177, 180–181, 184  
DTT buffer (recipe), 105, 113  
Dynein, 115, 116, 131. *See also* Motor proteins  
isolation of cytoplasmic dynein, 133–134  
separation of kinesin and dynein by sucrose gradient centrifugation, 134
- E**
- Electron microscopy  
for nuclear matrix analysis, 225  
for rough microsome purity analysis, 167  
Endocytosis, 27  
Endolysosomes, isolation of, 70  
Endoplasmic reticulum. *See* Rough microsomes  
Endosomes, 27–44  
advances in fractionation techniques for, 29  
endosome/lysosome system, 28f  
isolation by density gradient centrifugation (protocol), 36–39  
materials, 36  
method, 37–38  
continuous gradient, 37–38  
step gradient, 37  
recipes, 38  
isolation of macrophage endosomes by latex bead internalization and density gradient centrifugation (protocol), 40–44  
discussion, 42, 43f  
materials, 40–41  
method, 41–42  
discontinuous sucrose gradient preparation, 41–42  
harvesting cells, 41  
homogenizing cells, 41  
latex bead internalization, 41  
separation of organelles containing latex beads, 42  
recipes, 43–44  
troubleshooting, 42  
overview, 27–28  
proteome, 28–29  
Enzyme buffer for protoplasts (recipe), 162  
*Escherichia coli*, isolation of ribosomes by chromatography (protocol), 196–199  
materials, 196–197  
method, 197–198  
recipes, 198–199  
troubleshooting, 198  
ExoMir (Bioo Scientific), 61  
ExoQuick (System Biosciences), 61  
Exosomes, 59–63  
functions of, 60  
future study, 62  
properties of, 59–60  
purification methods, 60–61  
affinity-based capture, 61  
precipitation, 61  
size-based, 60–61  
ultracentrifugation, 60
- F**
- Fbp1p, 104f, 105  
FeDex, 72–75  
Ficoll, 70, 156  
Ficoll (20%) (recipe), 71  
Ficoll (25%) (recipe), 71  
5'-Nucleotidase activity, 8–9, 8t  
Flagellar axonemes, 139–140  
Fluorescent microscopy, for observation of microtubule gliding, 139  
FMG-1B, 116–117  
Fox3p, 104f, 105  
Fractionation. *See also specific protocols*  
assessing fractions, 3  
in GLUT4 storage vesicle preparation  
complete membrane fractionation of 3T3-L1 adipocytes, 86–91  
iodixanol gradient centrifugation to separate components of the low-density membrane fraction from 3T3-L1 adipocytes, 92–94  
preparation of a total membrane fraction from 3T3-L1 adipocytes (protocol), 79–81  
16K fractionation of 3T3-L1 adipocytes to produce a crude GLUT4-containing vesicle fraction (protocol), 82–85  
in lysosomal isolation from rat liver (protocol), 68–70  
overview, 2–3  
in peroxisome preparation, 102, 110  
subcellular fractionation of the brain, 49–52, 50f, 51t  
French press buffer for prokaryotic polysomes (recipe), 193  
French press buffer for prokaryotic 70S ribosomes (recipe), 193  
Fructose-1,6-bisphosphatase (Fbp1p), 104f, 105  
Fumarase activity buffer (recipe), 105  
Fumarase activity measurement, 104–105
- G**
- GalT activity, calculating, 143–144  
GalT assay mixture (recipe), 145  
Glass-Teflon homogenizer, 49–52, 152  
Glucose-6-phosphatase, 172  
Glucose transporter isoform 4 (GLUT4), 77–78  
GLUT4 storage vesicles (GSVs), isolation of, 77–95  
complete membrane fractionation of 3T3-L1 adipocytes (protocol), 86–91  
materials, 86–87  
method, 87–89  
immunoblotting, 89  
membrane fractionation, 87–89, 88f  
recipes, 90–91  
troubleshooting, 89–90  
iodixanol gradient centrifugation to separate components of the low-density membrane fraction from 3T3-L1 adipocytes (protocol), 92–94  
immunoblotting, 93, 94f  
materials, 92–93  
method, 93, 94f  
recipes, 94  
troubleshooting, 93–94  
preparation of a total membrane fraction from 3T3-L1 adipocytes (protocol), 79–81  
materials, 79–80  
method, 80–81  
concentrating soluble fractions by TCA precipitation, 80–81  
preparing total membrane fractions, 80  
recipe, 81  
troubleshooting, 81  
16K fractionation of 3T3-L1 adipocytes to produce a crude GLUT4-containing vesicle fraction (protocol), 82–85  
materials, 82–83  
method, 83–84  
concentrating the light membrane fraction by TCA precipitation, 83  
preparing 16K fractions, 83  
recipes, 85  
troubleshooting, 84  
Glycerol, 184–185  
in isolation of microtubules by assembly/disassembly methods (protocol), 118, 120–122  
Glycogen, 172  
Golgi isolation (protocol), 141–146  
discussion, 144–145  
materials, 141–142  
method, 142–144  
preparation of Golgi, 142–143  
yield determination, 143–144  
recipes, 145–146  
troubleshooting, 144  
Gradient buffer A (recipe), 106, 113  
Gradient buffer B (recipe), 106, 113  
Gradient buffers A–E for Golgi isolation (recipe), 145  
Gradient Master, 37–38
- H**
- HeLa cells, clathrin-coated vesicles (CCVs)  
isolation from  
advantages of using, 12  
limitations of method, 12–13  
protocol, 14–17  
materials, 14–15  
method, 15–16  
recipes, 16  
HEPES-sorbitol buffer (recipe), 162  
HES buffer (recipe), 81, 90, 94  
High-salt sucrose cushion buffer for chloroplast ribosomes (recipe), 194  
High-salt sucrose cushion buffer for prokaryotic ribosomes (recipe), 194  
Homogenization. *See also specific protocols*  
choice of method, 1–2  
in lysosomal isolation from rat liver (protocol), 68–70  
of mammalian cells (protocol), 32–35  
materials, 32–33  
method, 33–34  
protocol for cells that require hypotonic shock, 34  
standard protocol, 33–34  
recipes, 35  
troubleshooting, 34  
for mitochondria isolation, 148  
monitoring with phase-contrast microscopy, 33–34, 150  
Homogenization buffer, 2  
Homogenization buffer for endosome preparation (recipe), 35, 38, 43

- Homogenizers  
ball-bearing, 2  
choice of, 1–2  
Dounce, 1–2, 151, 152, 170, 176–177, 180–181, 184  
glass-Teflon, 49–52, 152  
Potter–Elvehjem, 1–2, 152–153, 170, 176, 184, 220
- Homogenizing buffer (recipe), 173
- Homogenizing medium for isolation of nuclei (recipe), 222
- Homogenizing medium for tissue culture cells (recipe), 185
- HPLC for exosome purification, 61
- HPLC for tubulin isolation, 129
- Hydrogen peroxide solution (recipe), 106, 113
- Hypertonic sucrose buffer (recipe), 222
- Hypotonic shock, 34
- I**
- ImageJ software, 9
- Immunoblotting  
analyzing GLUT4 distribution by, 84, 84f, 89, 89f, 93, 94f  
nuclear matrix analysis by, 230–231, 232, 232f  
for peroxisome markers, 104f, 105, 112f, 113  
with plasma membrane markers, 9
- Immunofluorescence, nuclear matrix protein analysis by, 229–230, 231–232, 232f
- In-gel digestion, 19
- In-solution digestion for proteome analysis of clathrin-coated vesicles (CCVs), 23–24
- Insulin, 77
- In vitro translation, concentration of rough microsomes for, 172–173
- Iodixanol gradient centrifugation to separate components of the low-density membrane fraction from 3T3-L1 adipocytes (protocol), 92–94  
immunoblotting, 93, 94f  
materials, 92–93  
method, 93, 94f  
recipes, 94  
troubleshooting, 93–94
- Iodoacetamide, 19, 24
- Ion exchange chromatography, separation of tubulin and microtubule-associated proteins by (protocol), 127–130  
discussion, 129  
materials, 127  
method, 128–129  
recipes, 129–130  
troubleshooting, 129
- Iron dextran, colloidal, 72–75
- Isotope tagging, 29
- iTRAQ, 29
- K**
- Kar2p, 104f, 105
- Kinesin, 115, 116, 131. *See also* Motor proteins  
isolation of, 132–133  
separation of kinesin and dynein by sucrose gradient centrifugation, 134
- L**
- Laemmli sample buffer (4×) (recipe), 218
- Laemmli sample buffer (LSB) (recipe), 85, 91, 94
- LAMP1, 27, 75
- Laser trap, 116–117
- Latex beads  
isolation of macrophage early and late endosomes by latex bead internalization and density gradient centrifugation (protocol), 40–44  
discussion, 42, 43f  
materials, 40–41  
method, 41–42  
recipes, 43–44  
troubleshooting, 42  
for observation of microtubule-based motor protein activity, 137, 139  
as perturbants, 29
- L-broth supplemented with 10 mM MgSO<sub>4</sub> (recipe), 199
- Lectin-magnetic beads for plasma membrane isolation (protocol), 5–9  
materials, 5–6  
method, 6–9, 6f, 8t  
ConA-magnetic beads preparation, 7  
determination of enrichment of plasma membrane proteins in eluted fraction, 8–9  
using immunoblots with plasma membrane markers, 9  
using 5′-nucleotidase activity, 8–9  
protein concentration determination, 8  
purification of plasma membranes from cells, 7–8, 8t  
schematic of isolation, 6f
- Lithium 3,5-diiodosalicylate, 223
- Liver  
lysosome isolation from rat liver (protocol), 67–71  
mitochondria isolation from (protocol), 152–154  
rough microsome preparation from rat liver (protocol), 168–174
- LTQ Orbitrap XL mass spectrometer, 20
- LysC, 24
- Lysis buffer with protease inhibitors (recipe), 106
- Lysosomal storage disorder, 65
- Lysosomes  
endosome/lysosome system, 28, 28f  
isolation from rat liver (protocol), 67–71  
discussion, 70  
materials, 67–68  
method, 68–70  
density-gradient centrifugation, 69  
isolation of endolysosomes, 70  
preparation of homogenate, 68–69  
recipes, 70–71  
overview, 65  
purification using supraparamagnetic iron oxide nanoparticles (protocol), 72–76  
discussion, 74–75  
materials, 72–73  
method, 73–74  
isolation of lysosomes, 73–74  
preparation of cell lysate, 73  
recipes, 75  
troubleshooting, 74
- Lyticase, 97, 100, 102, 110
- M**
- Macrophages, isolation of early and late endosomes by latex bead internalization and density gradient centrifugation (protocol), 40–44  
discussion, 42, 43f  
materials, 40–41  
method, 41–42  
discontinuous sucrose gradient preparation, 41–42  
harvesting cells, 41  
homogenizing cells, 41  
latex bead internalization, 41  
separation of organelles containing latex beads, 42  
recipes, 43–44  
troubleshooting, 42
- Magnetic beads  
lectin-magnetic beads for plasma membrane isolation (protocol), 5–9  
in phagosome isolation from tissue culture cells protocol, 55–57
- Mammalian cells, homogenization of (protocol), 32–35  
materials, 32–33  
method, 33–34  
protocol for cells that require hypotonic shock, 34  
standard protocol, 33–34  
recipes, 35  
troubleshooting, 34
- Mammalian reticulocyte ribosomes, cell extract preparation for, 191, 192
- Mammalian ribosome dissociation buffer (recipe), 209
- Mammalian ribosome homogenization buffer (recipe), 194
- Mammalian sucrose cushion buffer (recipe), 194
- Mammalian tissue ribosomes and polysomes cell extract preparation for, 191, 192  
density gradient centrifugation, 192  
dissociation of ribosomes into large and small subunits, 206–210
- Mannitol in mitochondria isolation protocols, 148
- MAPs. *See* Microtubule-associated proteins
- MAPs elution buffer (recipe), 129
- MAPs high-salt extraction solution (recipe), 125
- Mass spectrometer  
LTQ Orbitrap XL, 20  
Q Exactive, 24–25
- Mass spectrometry  
protein correlation profiling (PCP) by, 29–30  
for proteome analysis from clathrin-coated vesicles, 20, 24–25  
quantitative proteomics, 29–30
- MaxQuant software, 20, 25
- Membrane ion exchange chromatography for tubulin isolation, 129
- Metrizamide, 156
- Microsomes. *See also* Rough microsomes  
analysis of, 166–167  
isolation of, 165–166
- Microtubule-associated proteins (MAPs)  
isolation and analysis, 116  
isolation by salt extraction, 124–125  
isolation of microtubules by assembly/disassembly methods (protocol), 118–122

## Index

- Microtubule-associated proteins (MAPs)  
(*Continued*)  
separation of tubulin and  
microtubule-associated proteins  
by ion exchange chromatography  
(protocol), 127–130  
discussion, 129  
materials, 127  
method, 128–129  
recipes, 129–130  
troubleshooting, 129  
structure, 115
- Microtubules, 115–140. *See also*  
Microtubule-associated proteins  
(MAPs)  
gliding of, 138, 139–140  
isolation by assembly/disassembly methods  
(protocol), 118–122  
discussion, 121  
materials, 118–119  
method, 119–121  
brain tissue collection, 119  
isolation using buffer containing  
glycerol, 120–121  
isolation using buffer lacking  
assembly-promoting  
components, 119–120  
recipes, 121–122  
isolation of microtubule-based motor  
proteins by ATP release from  
paclitaxel-stabilized microtubules  
(protocol), 131–135  
discussion, 134  
materials, 131–132  
method, 132–134  
isolation of cytoplasmic dynein,  
133–134  
isolation of kinesin, 132–133  
separation of kinesin and dynein by  
sucrose gradient centrifugation,  
134  
recipes, 134–135  
isolation of microtubules and  
microtubule-associated proteins  
using paclitaxel (protocol),  
123–126  
materials, 123  
method, 124–125  
observation of microtubule-based motor  
protein activity (protocol),  
136–140  
discussion, 139–140  
bead movement on microtubule  
arrays nucleated by isolated  
centrosomes, 139  
gliding of flagellar axonemal  
microtubules whose plus ends  
can be identified in the  
microscope, 139–140  
gliding of microtubules with minus  
ends fluorescently labeled, 139  
materials, 136–137  
method, 137–138  
latex bead movement along  
microtubules, 137  
microtubule gliding, 138  
recipe, 140  
troubleshooting, 138  
polarity of, 131, 139  
structure, 115, 131
- Miescher, Friedrich, 211  
miRNA, in exosomes, 60
- Mitochondria isolation from cells and tissues,  
147–157  
isolation from animal tissue (protocol),  
152–154  
discussion, 154  
materials, 152  
method, 152–153  
recipe, 154  
troubleshooting, 153  
isolation from tissue culture cells (protocol),  
149–151  
discussion, 151  
materials, 149  
method, 150  
recipes, 151  
troubleshooting, 150–151  
markers for, 148  
purification by sucrose step density gradient  
centrifugation (protocol),  
155–157  
materials, 155  
method, 155–156  
recipes, 157  
troubleshooting, 156  
tips, 147–148
- Mitosis, 115  
Mono Q, 129
- Motor proteins, 115–117  
function of, 115  
isolation and analysis, 116  
isolation of microtubule-based motor  
proteins by ATP release from  
paclitaxel-stabilized microtubules  
(protocol), 131–135  
discussion, 134  
materials, 131–132  
method, 132–134  
isolation of cytoplasmic dynein,  
133–134  
isolation of kinesin, 132–133  
separation of kinesin and dynein by  
sucrose gradient centrifugation, 134  
recipes, 134–135  
observation of microtubule-based motor  
protein activity (protocol),  
136–140  
discussion, 139–140  
bead movement on microtubule  
arrays nucleated by isolated cen-  
trosomes, 139  
gliding of flagellar axonemal  
microtubules whose plus ends  
can be identified in the  
microscope, 139–140  
gliding of microtubules with minus  
ends fluorescently labeled, 139  
materials, 136–137  
method, 137–138  
latex bead movement along  
microtubules, 137  
microtubule gliding, 138  
recipe, 140  
troubleshooting, 138
- mRNA  
EDTA treatment of microsomes to strip  
membranes of endogenous  
ribosomes and mRNAs, 177  
in exosomes, 60  
MS homogenization buffer (1×) (recipe), 151,  
154, 157  
MS homogenization buffer (2.5×) (recipe), 151  
Multivesicular bodies (MVBs), 27
- N**  
NanoACQUITY UPLC system, 20  
Nitrogen cavitation, 2  
Nitrogen for cell disruption, 73  
NP-40, 215–217  
Nuclear matrix, 223–233  
functional analysis, approaches to, 224–226  
analysis of attached DNA, 225  
electron microscopy, 225  
proteomic analysis, 225  
protocol using cytoskeletal buffer,  
225–226  
schematic diagram, 224f  
overview, 223–224  
preparation for parallel microscopy and  
biochemical analyses (protocol),  
228–233  
discussion, 231–233  
considerations for immunoblotting  
analysis, 232  
considerations for  
immunofluorescence analysis,  
231–232  
method modifications, 232  
materials, 228–229  
method, 229–231, 230f  
flow diagram, 230f  
immunoblotting, 230–231  
immunofluorescence, 229–230  
recipes, 233  
troubleshooting, 231
- Nuclei isolation, 211–222  
analyzing isolated nuclei, 214  
history of, 211  
isolation of pure nuclei using the sucrose  
method (protocol), 219–222  
discussion, 221  
materials, 219–220  
method, 220–221  
recipes, 222  
key steps in, 212  
protocols compared, 212–213, 213f  
rapid isolation from cells in vitro (protocol),  
215–218  
discussion, 217–218  
materials, 215–216  
method, 216–217  
recipes, 218  
REAP method, 212–213, 213f, 215–218  
sucrose method, 213, 213f, 219–222
- Nycodenz, 65–66, 69–71, 98, 108, 156  
Nycodenz (20%) (recipe), 71  
Nycodenz (45%) (recipe), 71
- O**  
Oleic acid induction of yeast cells, 101  
OptiPrep, 92, 98, 100, 103, 105, 156
- P**  
Paclitaxel, 116  
isolation of microtubule-based motor  
proteins by ATP release from  
paclitaxel-stabilized microtubules  
(protocol), 131–135  
isolation of microtubules and  
microtubule-associated proteins  
using paclitaxel (protocol),  
123–126  
Paclitaxel (10 mM) (recipe), 125, 134

- Pancreas, rough microsome preparation from (protocol), 175–178
- PCP (protein correlation profiling), 29–30
- Pcs60p, 104f, 105
- Pearse, Barbara, 11
- Peptide extraction for proteome analysis from clathrin-coated vesicles, 20
- Peptide fractionation using SDB-RPS, 24
- Percoll, 156, 161
- Percoll gradient buffer (40%) (recipe), 162
- Percoll gradient buffer (85%) (recipe), 162
- Peroxisomes
- cytochrome *c* oxidase activity measurement, 103–104
  - isolating from yeast, 97–114
  - large-scale purification for preparative applications (protocol), 108–114
    - discussion, 112–113, 112f
    - materials, 108–109
    - method, 109–112
      - catalase activity measurement, 112
      - cultivating yeast cells, 109–110
      - density gradient centrifugation using Nycodenz gradients, 111–112
      - density gradient centrifugation using sucrose gradients, 110–111
      - obtaining organellar pellet, 110
      - spheroplast generation, 110
      - using subcellular fractionation to obtain organellar pellet, 110
    - recipes, 113–114
  - overview, 97
  - small-scale purification for analytical applications (protocol), 100–107
    - discussion, 105
    - materials, 100–101
    - method, 101–105
      - catalase activity measurement, 103
      - cultivating yeast cells, 101
      - cytochrome *c* oxidase activity measurement, 103–104
      - density gradient centrifugation, 102–103, 104
      - fumarase activity measurement, 104–105
      - measuring enzyme activity of fractions, 103–105
      - obtaining organellar pellet, 102
      - obtaining postnuclear supernatant, 102
      - spheroplast generation, 102
      - using subcellular fractionation to obtain organellar pellet, 102
    - recipes, 105–107
- Perturbants, 29
- Phagosome isolation from tissue culture cells (protocol), 55–58
  - discussion, 56–57
  - materials, 55–56
  - method, 56
  - recipes, 57
- Phase-contrast microscopy, monitoring homogenization with, 33–34, 150
- Phosphate-buffered saline (PBS) (recipe), 85, 185, 218, 221
- Phosphate-buffered saline for endosome preparation (recipe), 35, 43
- Phosphocellulose column buffer (recipe), 130
- Pig brain tissue, collecting, 119
- Plant cytoplasmic ribosomes and polysomes cell extract preparation for, 191, 192
- density gradient centrifugation, 192–193
  - isolating polysomes, 204–205
- Plant extraction buffer (recipe), 194
- Plant polysome sucrose gradient buffer (recipe), 205
- Plant protoplasts, chloroplast isolation from (protocol), 159–163
  - discussion, 162
  - materials, 159–160
  - method, 160–161
    - isolating chloroplasts, 161
    - isolating protoplasts, 160–161
  - recipes, 162–163
- Plant sucrose cushion buffer (recipe), 194
- Plasma membrane markers, using immunoblots with, 9
- Plasma membranes
- endosome network and, 27, 28f
  - lectin-magnetic beads for isolation (protocol), 5–9
    - ConA-magnetic beads preparation, 7
    - determination of enrichment of plasma membrane proteins in eluted fraction, 8–9
    - using immunoblots with plasma membrane markers, 9
    - using 5'-nucleotidase activity, 8–9
  - materials, 5–6
  - method, 6–9, 6f, 8t
  - protein concentration determination, 8
  - purification of plasma membranes from cells, 7–8, 8t
  - schematic of isolation, 6f
- Plastids, 162
- PME buffer (recipe), 121, 126, 135
- PME dynein buffer (recipe), 135
- PME-G buffer (recipe), 122
- PME sucrose solution (recipe), 126
- Polycellulose columns, for separation of tubulin and microtubule-associated proteins by ion exchange chromatography, 127–130
- Polysomes. *See also* Ribosomes
- isolation of ribosomes and polysomes (protocol), 189–195
    - materials, 189–190
    - method, 190–193
      - isolation of ribosomes and polysomes, 192–193
      - preparation of cell extracts, 190–192
    - recipes, 193–195
  - purification of polysomes (protocol), 203–205
    - materials, 203
    - method, 204–205
    - recipes, 205
- Porin, 104f, 105
- Postsynaptic density, 46, 46f, 51
- Potassium phosphate buffer (0.5 M, pH 6.7) (recipe), 146
- Potter–Elvehjem homogenizer, 1–2, 152–153, 170, 176, 184, 220
- Precipitation
- concentrating light membrane fraction by TCA precipitation, 83–84
  - concentrating soluble fractions by TCA precipitation, 80–81
  - for exosome purification, 61
- Prokaryotic bind/wash buffer (recipe), 198
- Prokaryotic elution buffer (recipe), 199
- Prokaryotic polysomes
- cell extract preparation for, 191, 192
  - isolation of, 204
- Prokaryotic polysome sucrose gradient buffer (recipe), 205
- Prokaryotic ribosome dissociation buffer (recipe), 210
- Prokaryotic ribosomes
- cell extract preparation for, 190–191
  - density gradient centrifugation, 192
  - dissociation of ribosomes into large and small subunits (protocol), 206–210
    - materials, 206–207
    - method, 207–209
    - recipes, 209–210
    - troubleshooting, 209
  - purification of 70S ribosomes (protocol), 200–202
    - materials, 200–201
    - method, 201, 202f
    - recipes, 202
    - troubleshooting, 202
- Prokaryotic storage buffer (recipe), 199
- Protease inhibitors, 142
- Protein Assay Dye Reagent Concentrate (Bio-Rad), 143
- Protein concentration determination, 8
- Protein correlation profiling (PCP), 29–30
- Proteins, in ribonucleoprotein complex, 187
- Proteome
- analysis of nuclear matrix, 225
  - endosome, 28–29
- Proteomics, quantitative methods in, 29–30
- Protoplasts, chloroplast isolation from plant (protocol), 159–163
  - discussion, 162
  - materials, 159–160
  - method, 160–161
    - isolating chloroplasts, 161
    - isolating protoplasts, 160–161
  - recipes, 162–163
- PTA/HCl (1% phosphotungstic acid/0.5 M HCl) (recipe), 146
- Puromycin, 167
- Q**
- Q Exactive mass spectrometer, 24–25
- R**
- Rab7, 28
- Rats
- lysosome isolation from rat liver (protocol), 67–71
  - rough microsome preparation from (protocol), 168–174
- REAP method of nuclei isolation, 212–213, 213f, 215–218
- Recipes
- $\beta$ -hexosaminidase substrate solution, 70
  - binding medium for phagosomes, 57
  - brain homogenization buffer, 52
  - buffer A for CCVs, 16, 21
  - buffer A for dog pancreas microsomes, 177
  - buffer A-FS, 16
  - buffer B for dog pancreas microsomes, 177
  - buffer C for stripping pancreatic rough microsome, 177
  - catalase activity buffer, 105, 113
  - chloroplast homogenization buffer, 193
  - chloroplast lysis buffer, 193

## Index

### Recipes (*Continued*)

- chloroplast ribosome dissociation buffer, 209
- colloidal iron dextran (FeDex), 75
- CS-mannitol buffer, 162
- CS-sucrose buffer, 162
- cytoskeletal (CSK) buffer, 233
- DTT buffer, 105, 113
- enzyme buffer for protoplasts, 162
- Ficoll (20%), 71
- Ficoll (25%), 71
- French press buffer for prokaryotic polysomes, 193
- French press buffer for prokaryotic 70S ribosomes, 193
- fumarase activity buffer, 105
- GalT assay mixture, 145
- gradient buffer A, 106, 113
- gradient buffer B, 106, 113
- gradient buffers A–E for Golgi isolation, 145
- HEPES-sorbitol buffer, 162
- HES buffer, 81, 90, 94
- high-salt sucrose cushion buffer for chloroplast ribosomes, 194
- high-salt sucrose cushion buffer for prokaryotic ribosomes, 194
- homogenization buffer for endosome preparation, 35, 38, 43
- homogenizing buffer, 173
- homogenizing medium for isolation of nuclei, 222
- homogenizing medium for tissue culture cells, 185
- hydrogen peroxide solution, 106, 113
- hypertonic sucrose buffer, 222
- Laemmli sample buffer (4×), 218
- Laemmli sample buffer (LSB), 85, 91, 94
- L-broth supplemented with 10 mM MgSO<sub>4</sub>, 199
- lysis buffer with protease inhibitors, 106
- mammalian ribosome dissociation buffer, 209
- mammalian ribosome homogenization buffer, 194
- mammalian sucrose cushion buffer, 194
- MAPs elution buffer, 129
- MAPs high-salt extraction solution, 125
- MS homogenization buffer (1×), 151, 154, 157
- MS homogenization buffer (2.5×), 151
- Nycodenz (20%), 71
- Nycodenz (45%), 71
- paclitaxel (10 mM), 125, 134
- Percoll gradient buffer (40%), 162
- Percoll gradient buffer (85%), 162
- phosphate-buffered saline (PBS), 85, 185, 218, 222
- phosphate-buffered saline for endosome preparation, 35, 43
- phosphocellulose column buffer, 130
- plant extraction buffer, 194
- plant polysome sucrose gradient buffer, 205
- plant sucrose cushion buffer, 194
- PME buffer, 121, 126, 135
- PME dynein buffer, 135
- PME-G buffer, 122
- PME sucrose solution, 126
- potassium phosphate buffer (0.5 M, pH 6.7), 146
- prokaryotic bind/wash buffer, 198
- prokaryotic elution buffer, 199
- prokaryotic polysome sucrose gradient buffer, 205
- prokaryotic ribosome dissociation buffer, 210
- prokaryotic storage buffer, 199
- PTA/HCl (1% phosphotungstic acid/0.5 M HCl), 146
- reduced cytochrome *c* solution, 106
- resuspension buffer for plant ribosomes, 195
- reticulocyte homogenization buffer, 195
- RIPA-SDS buffer, 218
- RNA loading dye, 210
- RSB hypo buffer, 151
- SD medium, 107, 114
- SDS-PAGE sample buffer (4×), 233
- sorbitol buffer for spheroplasts, 107, 114
- stage tip elution buffer 1, 25
- stage tip elution buffer 2, 25
- stage tip elution buffer 3, 25
- stage tip equilibration solution, 25
- stage tip MS buffer, 25
- STE fractionation buffer, 71
- STE sucrose buffer, 71
- STM fractionation buffer, 57, 75
- storage buffer, 210
- sucrose solutions for endosome purification, 38, 43
- sucrose step density gradient solutions, 157
- TAE, 210
- tight couples buffer, 202
- titanium oxysulfate solution, 107, 114
- TKM buffer for rough microsomes, 173
- TK20M buffer for rough microsomes, 173
- Valap sealant, 140
- WS solution for protoplasts, 163
- YNBO medium, 107, 114
- Reduced cytochrome *c* solution (recipe), 106
- Resuspension buffer for plant ribosomes (recipe), 195
- Reticulocyte homogenization buffer (recipe), 195
- Ribosomes, 187–210
  - dissociation of ribosomes into large and small subunits (protocol), 206–210
    - materials, 206–207
    - method, 207–209
    - recipes, 209–210
    - troubleshooting, 209
  - EDTA treatment of microsomes to strip membranes of endogenous ribosomes and mRNAs, 177
  - eukaryotic and prokaryotic, 187
  - isolation of ribosomes and polysomes (protocol), 189–195
    - materials, 189–190
    - method, 190–193
      - isolation of ribosomes and polysomes, 192–193
      - preparation of cell extracts, 190–192
    - recipes, 193–195
  - isolation of ribosomes by chromatography (protocol), 196–199
    - materials, 196–197
    - method, 197–198
    - recipes, 198–199
    - troubleshooting, 198
  - mitochondrial, 187
  - overview of isolation methods, 187–188, 188f
  - purification of polysomes (protocol), 203–205
    - materials, 203
  - method, 204–205
  - recipes, 205
  - purification of 70S ribosomes (protocol), 200–202
    - materials, 200–201
    - method, 201, 202f
    - recipes, 202
    - troubleshooting, 202
    - structure, 187
  - RIPA-SDS buffer (recipe), 218
  - RNA, in exosomes, 60
  - RNA loading dye (recipe), 210
  - RNase, 15, 172, 202, 209
  - RNase inhibitor, 171–172, 181–182
  - Rotors for centrifugation, 2–3
  - Rough microsomes
    - analysis of, 166–167
    - isolation of, 165–166
    - preparation from dog pancreas (protocol), 175–178
      - materials, 175–176
      - method, 176–177
        - EDTA treatment of microsomes to strip membranes of endogenous ribosomes and mRNAs, 177
        - preparation of crude microsomes, 176–177
    - recipes, 177–178
    - preparation from rat liver (protocol), 168–174
      - discussion, 172–173
      - materials, 168–169
      - method, 169–172
        - collection of fresh liver, 169
        - concentration of rough microsomes for in vitro translation, 171–172
        - preparation of high-speed supernatants, 171
        - preparation of membrane-bound polysomes, 171
        - preparation of rough microsome fraction, 170
        - processing of rough microsome fraction, 171–172
    - recipes, 173
    - preparation from tissue culture cells (protocol), 179–185
      - materials, 179–180
      - method, 180–184
        - centrifuging homogenate, 182–183
        - collecting and processing microsomes, 183–184
        - harvesting cells, 181
        - homogenizing cells, 181–182
        - pretreating the cells, 180
      - recipes, 185
      - troubleshooting, 184–185
    - RSB hypo buffer (recipe), 151

## S

- Saccharomyces cerevisiae*, isolation of peroxisomes from, 97–114
- Salt extraction, microtubule-associated proteins (MAPs) isolation by, 124–125
- SDB-RPS, peptide fractionation using, 24
- SD medium (recipe), 107, 114
- SDS-PAGE sample buffer (4×) (recipe), 233
- Shearing forces, 1–2
- Sodium chloride, for nuclear matrix estimation, 223, 225, 228, 230–232

- Sorbitol buffer for spheroplasts (recipe), 107, 114
- Sperm flagellar axonemes, 139–140
- Spheroplasts, yeast cell conversion to, 97–98, 100, 102, 110
- SPIONs. *See* Supraparamagnetic iron oxide nanoparticles
- Stage tip elution buffer 1 (recipe), 25
- Stage tip elution buffer 2 (recipe), 25
- Stage tip elution buffer 3 (recipe), 25
- Stage tip equilibration solution (recipe), 25
- Stage tip MS buffer (recipe), 25
- Starting material, choice of, 1
- STE fractionation buffer (recipe), 71
- STE sucrose buffer (recipe), 71
- STM fractionation buffer (recipe), 57, 75
- Storage buffer (recipe), 210
- Streptavidin magnetic beads, in plasma membrane isolation, 5–9, 6f, 8t
- Sucrose
- for density gradient centrifugation, 3
  - in endosome isolation protocols, 37–38, 41–42
  - in Golgi isolation protocol, 141–145
  - in mitochondria purification protocol, 155–157
  - in nuclei isolation, 219–222
  - in peroxisome purification protocol, 100–107, 110–111
  - in ribosome and polysome isolation, 188, 192–193, 200–210
  - in rough microsome isolation, 165–185
  - discontinuous sucrose gradient preparation, 41–42
  - separation of kinesin and dynein by sucrose gradient centrifugation, 134
- Sucrose solutions for endosome purification (recipe), 38, 44
- Sucrose step density gradient solutions (recipe), 157
- SulfoLink resin, 196–197
- Supraparamagnetic iron oxide nanoparticles (SPIONs), lysosome purification using (protocol), 72–76
- discussion, 74–75
  - materials, 72–73
  - method, 73–74
  - isolation of lysosomes, 73–74
  - preparation of cell lysate, 73
  - recipes, 75
  - troubleshooting, 74
- Synapse, 45, 46f
- Synaptic vesicles, preparation of, 49–52, 50f, 51t
- Synaptosomes, 45–53
- composition, 46f
  - as model system for studying synaptic physiology, 45–53
  - preparation (protocol), 49–52, 50f, 51t
  - materials, 49–50
  - method, 50–52
  - isolation of synaptic vesicles, 51–52
  - isolation of synaptosomes, 50–51
  - recipes, 52
  - schematic diagram, 50t
  - subcellular fractionation of the brain, 49–52, 50f, 51t
  - uses for synaptosomal preparations, 46–47
- T**
- TAE (recipe), 210
- Taxol, 116
- TCA precipitation, in GLUT4 storage vesicle preparation
- concentrating light membrane fraction, 83
  - concentrating soluble fractions, 80–81
- Tight couples buffer (recipes), 202
- Tissue culture cells. *See also specific protocols*
- clathrin-coated vesicles (CCVs) isolation from, 11–25
  - lysosome isolation from, 65–76
  - mitochondria isolation from (protocol), 149–151
  - nuclei isolation protocols, 215–222
  - REAP method, 215–218
  - sucrose method, 219–222
  - phagosome isolation from (protocol), 55–58
  - rough microsome preparation from (protocol), 179–185
- Titanium oxysulfate solution (recipe), 107, 114
- TK20M buffer for rough microsomes (recipe), 173
- TKM buffer for rough microsomes (recipe), 173
- Total Exosome Isolation reagents (Life Technologies), 61
- Triton WR1339, 65
- Triton X-100
- for nuclear matrix, 225, 228, 230
  - for nuclei isolation, 217–218, 219–221
  - for rough microsome isolation, 172
- Trypsin, for proteome analysis of clathrin-coated vesicles (CCVs)
- in-gel digestion, 19
  - in-solution digestion, 24
- Tubulin, 115, 116, 118. *See also* Microtubules
- binding to paclitaxel, 123–124
  - fluorescent, 139
  - isolation in buffer containing glycerol, 120–121
  - observation of microtubule-based motor protein activity (protocol), 136–140
  - separation of tubulin and microtubule-associated proteins by ion exchange chromatography (protocol), 127–130
  - discussion, 129
  - materials, 127
  - method, 128–129
  - recipes, 129–130
  - troubleshooting, 129
- U**
- Ultracentrifugation for exosome purification, 60
- Ultrafiltration for exosome purification, 60–61
- V**
- Valap sealant (recipe), 140
- Vn peptides, 61
- W**
- WS solution for protoplasts (recipe), 163
- X**
- XCalibur software, 24
- Y**
- Yeast, isolation of peroxisomes from, 97–114
- YNBO medium (recipe), 107, 114
- Z**
- Zymolase, 98