

# Index

## A

- Adenine, radiolabeled, 80
- Adenosine 3',5'-cyclic monophosphate, *see* cyclic AMP
- Adenosine triphosphosphate, *see* ATP
- Adenylyl cyclase, 63–76, 87
  - calculation of activity, 70
  - receptors coupled to, 79
- ADP-ribosylating buffer, 54
- Adrenal glands,
  - cyclic AMP binding protein, 81, 85
  - inositol phosphates binding protein, 169
- Affinity chromatography, 18, 19
  - domoic acid, 18, 19, 21
  - cyclic AMP-dependent protein kinase, *see* PKA
- AMPA binding sites, 18
- Arachidonic acid, radiolabeled, 191
- ATP regenerating system, 65, 66, 72
- Autofluorescence, 207, 209
- Autoradiography, 25–38, 248
  - biochemical studies, 29
  - preincubation and labeling, 29
  - qualitative, 30
  - qualitative, high resolution, 31
  - quantitative, 32
  - quantitative, analysis, 34
  - radioligand preparation, 29
  - section cutting, 28
  - slide preparation, 26, 27
  - tissue preparation, 26, 28

## B

- $\beta$ -adrenoceptor antagonist radioligands, 25
- $\beta$ -adrenoceptors, 25
- $\beta$ -eccsin, 217

## C

- Caffeine, 225
- Calcium/calmodulin-stimulated protein kinase II,
  - see* CaM-PK II
- Calcium ion contamination, removal, 218
- <sup>45</sup>Calcium ion fluxes, 215, 219–222
- Calcium ion fluxes, permeabilized cells, 215–225
- Calcium ion imaging in single cells, 229–237
  - calibration, 236
  - graphical analysis, 236
  - image capture, 235, 236
  - image digitization, 234
  - image ratioing, 236
  - image storage, 237
  - pseudocoloring, 234
- Calcium ion release, inositol trisphosphate mediated, 222
- Calcium ion-sensitive electrodes, 225
- Calcium ion uptake, 222
- Calcium ionophore, 206
- Calcium ions,
  - concentration in cell monolayers, 207

- concentration in cell populations, 203
  - concentration in cell suspensions, 205–207
  - CaM-PK II, 239–259
    - activity, 240
    - antibody binding, 252
    - autophosphorylation, 240, 241, 248, 249, 258
    - calmodulin binding assay, 253
    - levels, 242
    - peptide assay, 249, 251
    - Western blot, 252
  - Cell harvester, 5
  - Centrifugation, separation of radioligands, 5
  - Ceramide phosphate, 200
  - Chemography, positive, 35
  - Cheng-Prusoff, 11
  - CHO-NAX2 cells, 208
  - Coomassie brilliant blue, 32
  - Coverslip holders, 209, 210
  - Curve fitting, 14, 86, 173
  - Cyclic AMP, 79–88, 63
    - enzyme immunoassay, 92, 95
    - extraction from biological samples, 98, 99
    - mass measurements, 91–103
    - radioimmunoassay, 91, 93–95, 100, 101
      - acetylation, 100
      - antibodies, 93, 95
      - cyclic AMP radioiodination, 94
      - nonacetylation, 100
      - preparation of immunogens, 93
      - scintillation proximity, 101
      - separation methods, 96
    - radioreceptor assay, 81, 85
  - Cyclic GMP, 107–111
    - binding assay, 126
  - Cyclic GMP-dependent protein kinase, *see* G-kinase
  - Cyclic nucleotide phosphodiesterases, *see* PDE
- D**
- DAG, 189–200
    - chromatographic assays, 196, 197
    - derived from PLC or PLD, 197–199
    - kinase, 195
    - mass assay, 195, 275
    - radioisotope prelabeling assay, 193–195
  - DDT1 MF2 cells, 209
  - Deacylation of lipid extract, 171
  - Densitometry, 32, 33, 245, 248
  - Deoxycholate, 179, 181, 185
    - depletion, 116, 117
  - Diacylglycerol, *see* DAG
  - Dowex/alumina sequential chromatography, 68, 70, 73, 81, 83, 109, 110
  - Dowex column chromatography, 67
- E**
- Electroporation, 219
  - Excitatory amino acid receptor, 17
- F**
- Filtration separation of radioligands, 5
  - Fluo-3, 215, 216, 223
  - Fluorescence microscope, 232
  - Fluorescence ratios, calibration, 209
  - Fluorescence spectrophotometer (fluorimeter), 205, 217
  - Forskolin, 73
  - Freon-octylamine, 154, 156
  - Fura-2, 230
  - Fura-2, dual wavelength recording, 204
  - Fura-2AM, 231, 235
- G**
- G-kinase, 123–126
    - preparation from bovine lung, 124
    - substrates, 123, 124

G-proteins, 8, 51, 63, 177  
  anti-G-protein antisera, 57  
  anti-G-protein toxins, 57  
Glycerol, radiolabeled, 191  
Glycerophosphoinositols, 162  
Grynkiewicz equation, 206, 218,  
  231  
GTP analogs, 178  
GTPase, 51–60  
  calcium channels, 52, 60  
  calculation of GTP hydrolysis, 57  
  kinetic analysis, 55  
Guanine nucleotide binding proteins,  
  *see* G-proteins  
Guanine, radiolabeled, 109

## H

Heparin, 172  
High-performance liquid chromatog-  
raphy, *see* HPLC  
HPLC separation of inositol phos-  
phates, 151–163  
Hybridization stringency, 42

## I

Inositol phosphate isomers, 151, 152  
Inositol phosphates binding proteins,  
  169  
Inositol phosphates,  
  HPLC separation, 151–163  
  elution gradients, 156–161  
  sample preparation, 155, 156  
Inositol phosphates, mass measure-  
ments, 167–175  
Inositol phosphates, radiolabeled,  
  155  
Inositol, radiolabeled, 155  
*In-situ* hybridization, 41–49  
  hybridization, 45, 46  
  probe preparation, 44, 46  
  tissue preparation, 44, 45

## K

Kainate/AMPA receptor, 18  
Kemptide, 114

## L

Lineweaver-Burk plots, 55  
Lyso-phosphatidic acid, 200

## M

MagiCal, 230  
Malantide, 114  
Metalloporphyrin, 286

## N

N1E-115 neuroblastoma cells, 205  
Neuronal membranes, 54  
Neutrophils, isolation, 263, 264  
NG108-15 cells, 68  
Nitric oxide, 285–297  
  bioassay, 289  
  chemiluminescence, 287, 288  
  cyclic GMP, 290  
  electrochemical sensor, 286  
  electron paramagnetic resonance  
  spectroscopy, 295  
  gas chromatography–mass spec-  
  trometry, 288, 291  
  Griess reaction, 291  
  high-performance capillary  
  electrophoresis, 292  
  Malinski electrode, 286  
  nitrate and nitrite, 290  
  S-nitrosothiol measurement, 294  
  spin traps, 295  
Nonequilibrium binding, 11  
Nonspecific binding, 3  
Northern analysis, 48  
Nucleotide triphosphatase inhibitors,  
  53, 59  
Nucleotides, radioactive, 42

**O**

Oligodeoxyribonucleotides, 41, 42

**P**

Palmitic acid, radiolabeled, 191

Partisil/partisphere HPLC columns,  
155

PDE, 64, 66

PDE activity,

cell homogenates, 138–140

tracheal smooth muscle, 140–145

PDE inhibitors, 66, 72, 113, 144, 145

PDE isoenzyme activity, 129–147

PDE isoenzymes,

separation, 130

by affinity chromatography,

134, 135

by ion-exchange chromatography,  
134

Permeabilization of cells, 219

Peroxidase-related sites, 35

Phorbol ester, 263

Phosphatidate, Coomassie blue  
staining, 277

Phosphatidate phosphohydrolase, 189

Phosphatidic acid, 189, 200

Phosphatidyl alcohols, 198

Phosphatidylbutanol, 272

Phosphatidylcholine, 189, 190, 271

Phosphatidylinositol 4,5-

bisphosphate, *see* PIP<sub>2</sub>

Phosphoinositide kinases, 184

Phosphoinositide-specific phospholipase C, *see* PLC

Phosphoinositides, 151, 152

Phospholipase A<sub>2</sub>, *see* PLA<sub>2</sub>

Phospholipase D, *see* PLD

Phosphorylated lipids, quantification  
by TLC, 276

PIP<sub>2</sub>,

extraction and hydrolysis, 171

mass assay, 171

PKA, 63,

PKA activity ratio assay, 113–121  
measurement of ATP depletion,  
116

measurement of ATP specific  
activity, 116

tissue sample preparation, 114,  
115

PKC, 261–270

inhibitors, 267

substrate phosphorylation, 266,  
267

translocation,

phorbol ester induced, 263, 264

visualization with fluorescent  
probe, 264, 266

Western blot, 265

PLA<sub>2</sub> activity, 279–283

fatty acid release, 281

lysophospholipid production, 282  
substrates, 280

PLC, 167, 189

activity,

<sup>3</sup>H-inositol prelabeled mem-  
branes, 184

<sup>3</sup>H-inositol prelabeled tissue  
slices, 179, 180

brain membranes, 177–187

exogenously added <sup>3</sup>H-  
phosphoinositides, 179, 180,  
182

PLD, 189, 271–278

choline and choline phosphate  
generation, 274

transphosphatidylation assay, 272

Polyethylenimine, 5

G-kinase assay, 124, 126

Protease digestion, 247

Protease inhibitors, 147

Proteases, 23

Protein determination, 32

Protein kinase C, *see* PKC

Purification of receptors, 17, 19

**Q**

Quin-2, 203

**R**

Radioligand binding, 1–15  
  choice of radioligand, 2  
  data analysis, 14  
  definition of receptors, 13  
  depletion of radioligand, 10  
  impure radioligands, 7  
  incomplete separation of  
    radioligand, 10  
  ligand instability, 8  
  radioligand dissociation, 10  
  receptor instability, 9  
  receptor preparation, 4  
Reconstitution of receptors, 22  
RNase, 47

**S**

Saponin, 217  
Scintillation cocktail, 155

SDS-PAGE, 18, 23, 245  
SH-SY5Y neuroblastoma cells, 215  
Sodium dodecyl sulfate-polyacryla-  
  mide gel electrophoresis, *see*  
  SDS-PAGE  
Sodium fluoride, 73  
Solubilization of receptors, 5, 17, 19,  
  21  
Specific activity, 7  
Specific binding, 6  
Sulphydryl reagents, 147  
Synapsin I, 242

**T**

TARDIS, 230  
Terminal deoxynucleotidyl  
  transferase, 42  
Tyrosine hydroxylase, 242

**X**

Xenopus brain, 18, 19  
Xenopus brain membranes, 19, 21