Contents

List of Figures, xix
List of Color Plates, xxiii
List of Protocols, xxv
Preface and Acknowledgements, xxvii
Abbreviations, xxix

1. Introduction, 1
   1.1. Historical Background, 1
   1.2. Advantages of Tissue Culture, 6
       1.2.1. Control of the Environment, 6
       1.2.2. Characterization and Homogeneity of Samples, 6
       1.2.3. Economy, Scale, and Mechanization, 6
       1.2.4. In vitro Modeling of In vivo Conditions, 7
   1.3. Limitations, 7
       1.3.1. Expertise, 7
       1.3.2. Quantity, 7
       1.3.3. Dedifferentiation and Selection, 8
       1.3.4. Origin of Cells, 8
       1.3.5. Instability, 8
   1.4. Major Differences In vitro, 8
   1.5. Types of Tissue Culture, 8

2. Biology of Cultured Cells, 11
   2.1. The Culture Environment, 11
   2.2. Cell Adhesion, 11
       2.2.1. Cell Adhesion Molecules, 11

   2.2.2. Intercellular Junctions, 12
   2.2.3. Extracellular Matrix, 13
   2.2.4. Cytoskeleton, 14
   2.2.5. Cell Motility, 14

2.3. Cell Proliferation, 15
   2.3.1. Cell Cycle, 15
   2.3.2. Control of Cell Proliferation, 15

2.4. Differentiation, 16
   2.4.1. Maintenance of Differentiation, 17
   2.4.2. Dedifferentiation, 17

2.5. Cell Signaling, 17

2.6. Energy Metabolism, 19

2.7. Origin of Cultured Cells, 20
   2.7.1. Initiation of the Culture, 21
   2.7.2. Evolution of Cell Lines, 21
   2.7.3. Senescence, 22
   2.7.4. Transformation and the Development of Continuous Cell Lines, 22

3. Laboratory Design, Layout, and Equipment, 25
   3.1. Layout, Furnishing, and Services, 25
       3.1.1. Requirements, 25
       3.1.2. Services, 28
       3.1.3. Ventilation, 30
   3.2. Layout, 30
       3.2.1. Sterile Handling Area, 30
       3.2.2. Laminar Flow, 30
       3.2.3. Service Bench, 30
       3.2.4. Quarantine and Containment, 30
4. Equipment and Materials, 37

4.1. Requirements of a Tissue Culture Laboratory, 37

4.2. Aseptic Area, 37
4.2.1. Laminar-Flow Hood, 37
4.2.2. Service Carts, 41
4.2.3. Sterile Liquid Handling—Pipetting and Dispensing, 41
4.2.4. Inverted Microscope, 45
4.2.5. CCD Camera and Monitor, 46
4.2.6. Dissecting Microscope, 46
4.2.7. Centrifuge, 47
4.2.8. Cell Counting, 47

4.3. Incubation and Culture, 47
4.3.1. Incubator, 47
4.3.2. Humid CO₂ Incubator, 48
4.3.3. Temperature Recorder, 48
4.3.4. Roller Racks, 49
4.3.5. Magnetic Stirrer, 50
4.3.6. Culture Vessels, 50

4.4. Preparation and Sterilization, 50
4.4.1. Washup, 50
4.4.2. Preparation of Media and Reagents, 51
4.4.3. Sterilization, 52

4.5. Storage, 53
4.5.1. Consumables, 53
4.5.2. Refrigerators and Freezers, 54
4.5.3. Cryostorage Containers, 55
4.5.4. Controlled-Rate Freezer, 55

4.6. Supplementary Laboratory Equipment, 55
4.6.1. Computers and Networks, 55
4.6.2. Upright Microscope, 55
4.6.3. Low-Temperature Freezer, 56
4.6.4. Confocal Microscope, 56
4.6.5. PCR Thermal Cycler, 56

4.7. Specialized Equipment, 56
4.7.1. Microinjection Facilities, 56
4.7.2. Colony Counter, 56
4.7.3. Centrifugal Elutriator, 56
4.7.4. Flow Cytometer, 56

5. Aseptic Technique, 57

5.1. Objectives of Aseptic Technique, 57
5.1.1. Risk of Contamination, 57
5.1.2. Maintaining Sterility, 57

5.2. Elements of Aseptic Environment, 58
5.2.1. Laminar Flow, 58

5.2.2. Quiet Area, 60
5.2.3. Work Surface, 61
5.2.4. Personal Hygiene, 61
5.2.5. Reagents and Media, 61
5.2.6. Cultures, 61

5.3. Sterile Handling, 61
5.3.1. Swabbing, 61
5.3.2. Capping, 63
5.3.3. Flaming, 63
5.3.4. Handling Bottles and Flasks, 64
5.3.5. Pipetting, 64
5.3.6. Pouring, 65

5.4. Standard Procedure, 65
Protocol 5.1. Aseptic Technique in Vertical Laminar Flow, 65
Protocol 5.2. Working on the Open Bench, 67
Protocol 5.3. Handling Dishes or Plates, 69

5.5. Apparatus and Equipment, 69
5.5.1. Incubators, 69
5.5.2. Boxed Cultures, 70
5.5.3. Gassing with CO₂, 70

6. Safety, Bioethics, and Validation, 71

6.1. Laboratory Safety, 71
6.2. Risk Assessment, 71
6.3. Standard Operating Procedures, 73
6.4. Safety Regulations, 73
6.5. General Safety, 74
6.5.1. Operator, 74
6.5.2. Equipment, 74
6.5.3. Glassware and Sharp Items, 74
6.5.4. Chemical Toxicity, 76
6.5.5. Gases, 76
6.5.6. Liquid Nitrogen, 76
6.5.7. Burns, 78

6.6. Fire, 78
6.7. Ionizing Radiation, 78
6.7.1. Ingestion, 78
6.7.2. Disposal of Radioactive Waste, 78
6.7.3. Irradiation from Labeled Reagents, 78
6.7.4. Irradiation from High-Energy Sources, 79

6.8. Biohazards, 79
6.8.1. Levels of Biological Containment, 79
6.8.2. Microbiological Safety Cabinets (MSCs), 79
6.8.3. Human Biopsy Material, 79
6.8.4. Genetic Manipulation, 84
6.8.5. Disposal of Biohazardous Waste, 85
6.8.6. Fumigation, 85

6.9. Bioethics, 86
6.9.1. Animal Tissue, 86
6.9.2. Human Tissue, 86
6.10. Quality Assurance, 87
   6.10.1. Procedures, 87
   6.10.2. Quality Control (QC), 87

6.11. Validation, 87
   6.11.1. Authentication, 87
   6.11.2. Provenance, 88
   6.11.3. Contamination, 88

7. Culture Vessels and Substrates, 89

7.1. The Substrate, 89
   7.1.1. Attachment and Growth, 89
   7.1.2. Common Substrate Materials, 89
   7.1.3. Alternative Substrates, 90

7.2. Treated Surfaces, 90
   7.2.1. Substrate Coating, 90

Protocol 7.1. Preparation of ECM, 91
   7.2.2. Feeder Layers, 91
   7.2.3. Nonadhesive Substrates, 91

7.3. Choice of Culture Vessel, 91
   7.3.1. Cell Yield, 93
   7.3.2. Suspension Culture, 93
   7.3.3. Venting, 94
   7.3.4. Sampling and Analysis, 94
   7.3.5. Uneven Growth, 95
   7.3.6. Cost, 96

7.4. Specialized Systems, 96
   7.4.1. Permeable Supports, 96
   7.4.2. Three-dimensional Matrices, 97

8. Defined Media and Supplements, 99

8.1. Development of Media, 99

8.2. Physicochemical Properties, 99
   8.2.1. pH, 99

Protocol 8.1. Preparation of pH Standards, 100
   8.2.2. CO₂ and Bicarbonate, 100
   8.2.3. Buffering, 101
   8.2.4. Oxygen, 105
   8.2.5. Osmolarity, 106
   8.2.6. Temperature, 106
   8.2.7. Viscosity, 107
   8.2.8. Surface Tension and Foaming, 107

8.3. Balanced Salt Solutions, 107

8.4. Complete Media, 107
   8.4.1. Amino Acids, 108
   8.4.2. Vitamins, 108
   8.4.3. Salts, 108
   8.4.4. Glucose, 108
   8.4.5. Organic Supplements, 108
   8.4.6. Hormones and Growth Factors, 109
   8.4.7. Antibiotics, 109

8.5. Serum, 109
   8.5.1. Protein, 109
   8.5.2. Growth Factors, 111
   8.5.3. Hormones, 111
   8.5.4. Nutrients and Metabolites, 111
   8.5.5. Lipids, 111
   8.5.6. Minerals, 111
   8.5.7. Inhibitors, 111

8.6. Selection of Medium and Serum, 111
   8.6.1. Batch Reservoir, 112
   8.6.2. Testing Serum, 113
   8.6.3. Heat Inactivation, 114

8.7. Other Supplements, 114
   8.7.1. Amino Acid Hydrolysates, 114
   8.7.2. Embryo Extract, 114
   8.7.3. Conditioned Medium, 114

9. Serum-Free Media, 115

9.1. Disadvantages of Serum, 115

9.2. Advantages of Serum-Free Media, 121
   9.2.1. Definition of Standard Medium, 121
   9.2.2. Selective Media, 121
   9.2.3. Regulation of Proliferation and Differentiation, 121

9.3. Disadvantages of Serum-Free Media, 122

9.4. Replacement of Serum, 122
   9.4.1. Commercially Available Serum-Free Media, 122
   9.4.2. Serum Substitutes, 122
   9.4.3. Serum-Free Subculture, 123
   9.4.4. Hormones, 123
   9.4.5. Growth Factors, 123
   9.4.6. Nutrients in Serum, 124
   9.4.7. Proteins and Polyamines, 124
   9.4.8. Viscosity, 124

9.5. Selection of Serum-Free Medium, 124
   9.5.1. Cell or Product Specificity, 124
   9.5.2. Adaptation to Serum-Free Media, 124

9.6. Development of Serum-Free Medium, 124

9.7. Preparation of Serum-Free Medium, 129

9.8. Animal Protein-Free Media, 129

9.9. Conclusions, 132

10. Preparation and Sterilization, 133

10.1. Preparation of Reagents and Materials, 133

10.2. Sterilization of Apparatus and Liquids, 133

10.3. Apparatus, 134
   10.3.1. Glassware, 134

Protocol 10.1. Preparation and Sterilization of Glassware, 135
11. Primary Culture, 163

11.1. Initiation of a Primary Cell Culture, 163
   11.1.1. Enzymes Used in Disaggregation, 163
   11.1.2. Common Features of Disaggregation, 164

11.2. Isolation of the Tissue, 164
   11.2.1. Mouse Embryo, 164
   11.2.2. Chick Embryo, 166
   11.2.3. Human Biopsy Material, 168
   11.2.4. Handling Human Biopsies, 170

11.3. Types of Primary Culture, 170
   11.3.1. Primary Explantation, 170
   11.3.2. Enzymatic Disaggregation, 173
   11.3.3. Warm Trypsin, 173
   11.3.4. Trypsinization with Cold Preexposure, 175
   11.3.5. Trypsin, 176
   11.3.6. Chick Embryo Organ Rudiments, 177
   11.3.7. Other Enzymatic Procedures, 181
   11.3.8. Collagenase, 181
   11.3.9. Mechanical Disaggregation, 183
   11.3.10. Separation of Viable and Nonviable Cells, 184
   11.3.11. Primary Culture in Summary, 186

12. Subculture and Cell Lines, 187

12.1. Subculture and Propagation, 187
   12.1.1. Cross-contamination and Misidentification, 187
   12.1.2. Mycoplasma Contamination, 191
   12.1.3. Terminology, 191
   12.1.4. Naming a Cell Line, 192
   12.1.5. Culture Age, 192

12.2. Choosing a Cell Line, 193

12.3. Routine Maintenance, 193
   12.3.1. Significance of Cell Morphology, 193
12.4. Subculture, 196
12.4.1. Criteria for Subculture, 197
12.4.2. Typical Subculture Protocol for Cells Grown as a Monolayer, 199
12.4.3. Subculture of Monolayer Cells, 199
12.4.4. Cell Concentration at Subculture, 202
12.4.5. Propagation in Suspension, 202
12.4.6. Subculture of Cells Growing in Suspension, 202
12.4.7. Subculture of Suspension Cells, 203
12.4.8. Standardization of Culture Conditions, 204
12.4.9. Use of Antibiotics, 205
12.4.10. Maintenance Records, 206

13. Cloning and Selection, 207
13.1.2. Stimulation of Plating Efficiency, 209
13.1.2.1. Conditions That Improve Clonal Growth, 211
13.1.2.2. Conditioned Medium, 212
13.1.2.3. Preparation of Conditioned Medium, 212
13.1.2.4. Feeder Layers, 213
13.1.2.5. Preparation of Feeder Layers, 213
13.2. Suspension Cloning, 214
13.2.1. Cloning in Agar, 214
13.2.2. Cloning in Methocel, 217
13.3. Isolation of Clones, 218
13.3.1. Isolation of Clones with Cloning Rings, 218
13.3.2. Isolating Cell Colonies by Irradiation, 219
13.3.3. Other Isolation Techniques for Monolayer Clones, 220
13.3.4. Suspension Clones, 221
13.3.5. Isolation of Suspension Clones, 221
13.4. Replica Plating, 221
13.5. Selective Inhibitors, 221
13.6. Isolation of Genetic Variants, 223
13.7. Methotrexate Resistance and DHFR Amplification, 223
13.8. Interaction with Substrate, 224
13.8.1. Selective Adhesion, 224
13.8.2. Selective Detachment, 224
13.8.3. Nature of Substrate, 225
13.8.4. Selective Feeder Layers, 225
13.8.5. Selection by Semisolid Media, 225

14. Cell Separation, 227
14.1.1. Cell Separation by Centrifugation on a Density Gradient, 227
14.2. Cell Size and Sedimentation Velocity, 230
14.2.1. Unit Gravity Sedimentation, 230
14.2.2. Centrifugal Elutriation, 230
14.3. Antibody-Based Techniques, 232
14.3.1. Immune Panning, 232
14.3.2. Magnetic Sorting, 233
14.3.3. Magnetic Sorting (MACS), 234
14.4. Fluorescence-Activated Cell Sorting, 234
14.5. Other Techniques, 236

15. Characterization, 239
15.1. The Need for Characterization, 239
15.2. Authentication, 239
15.3. Record Keeping and Provenance, 240
15.4. Parameters of Characterization, 240
15.4.1. Species Identification, 240
15.4.2. Lineage or Tissue Markers, 241
15.4.3. Unique Markers, 242
15.4.4. Transformation, 242
15.5. Cell Morphology, 242
15.5.1. Microscopy, 247
15.5.2. Staining, 248
15.5.3. Staining with Giemsa, 249
15.5.4. Staining with Crystal Violet, 249
15.5.5. Culture Vessels for Cytology: Monolayer Cultures, 250
15.5.6. Preparation of Suspension Culture for Cytology, 250
15.5.7. Preparation of Suspension Cells for Cytology by Cyto centrifuge, 251
15.5.8. Filtration Cytology, 251
15.5.9. Photomicrography, 252
15.6. Confoal Microscopy, 253
15.7. Chromosome Content, 253
   Protocol 15.7. Chromosome Preparations, 253
      15.7.1. Chromosome Banding, 255
      15.7.2. Chromosome Analysis, 256
15.8. DNA Analysis, 256
   15.8.1. DNA Hybridization, 256
   15.8.2. DNA Fingerprinting, 257
   15.8.3. DNA Profiling, 258
   Protocol 15.8. DNA STR Profiling of Cell Lines, 259
15.9. RNA and Protein Expression, 261
15.10. Enzyme Activity, 261
      15.10.1. Isoenzymes, 262
      15.10.2. Isoenzyme Electrophoresis with
               Authentikit, 263
   Protocol 15.9. Isoenzyme Analysis, 263
15.11. Antigenic Markers, 267
      15.11.1. Immunostaining, 267
   Protocol 15.10. Indirect Immunofluorescence, 267
      15.11.2. Immunofluorescence, 268
15.12. Differentiation, 268

16. Differentiation, 269
16.1. Expression of the In vivo Phenotype, 269
   16.1.1. Dedifferentiation, 269
   16.1.2. Lineage Selection, 269
16.2. Stages of Differentiation, 270
16.3. Proliferation and Differentiation, 270
16.4. Commitment and Lineage, 270
16.5. Stem Cell Plasticity, 271
16.6. Markers of Differentiation, 272
   Protocol 15.10. Induction of Differentiation, 272
      16.6.1. Induction of Differentiation, 272
16.7. Induction of Differentiation, 272
   16.7.1. Cell Interaction, 273
   16.7.2. Systemic Factors, 274
   16.7.3. Cell–Matrix Interactions, 277
   16.7.4. Polarity and Cell Shape, 277
   16.7.5. Oxygen Tension, 277
16.8. Differentiation and Malignancy, 278
16.9. Practical Aspects, 278

17. Transformation and Immortalization, 279
17.1. Role in Cell Line Characterization, 279
17.2. What is Transformation?, 279
17.3. Genetic Instability and Heterogeneity, 279
   17.3.1. Genetic Instability, 279
   17.3.2. Chromosomal Aberrations, 281
17.4. Immortalization, 281
   17.4.1. Control of Senescence, 282
17.4.2. Immortalization with Viral Genes, 283
17.4.3. Immortalization of Human Fibroblasts, 283
   Protocol 17.1. Fibroblast Immortalization, 284
      17.4.4. Telomerase–Induced Immortalization, 287
   Protocol 17.2. Immortalization of Human Stem and
      Primary Cells by Telomerase, 287
      17.4.5. Lymphocyte Immortalization, 290
      17.4.6. Transgenic Mouse, 290
17.5. Aberrant Growth Control, 290
   17.5.1. Anchorage Independence, 290
   17.5.2. Contact Inhibition, 291
   Protocol 17.3. Density Limitation of Cell Proliferation, 291
      17.5.3. Serum Dependence, 292
      17.5.4. Oncogenes, 293
17.6. Tumorigenicity, 293
   17.6.1. Malignancy, 293
   17.6.2. Tumor Transplantation, 293
   17.6.3. Invasiveness, 294
   17.6.4. Angiogenesis, 294
   Protocol 17.4. In vitro Angiogenesis Assay, 295
      17.6.5. Plasminogen Activator, 297

18. Contamination, 299
18.1. Sources of Contamination, 299
   18.1.1. Operator Technique, 299
   18.1.2. Environment, 299
   18.1.3. Use and Maintenance of Laminar-Flow Hood, 299
   18.1.4. Humid Incubators, 300
   Protocol 18.1. Cleaning Incubators, 300
      18.1.5. Cold Stores, 301
      18.1.6. Sterile Materials, 301
      18.1.7. Imported Cell Lines and Biopsies, 301
      18.1.8. Quarantine, 301
18.2. Types of Microbial Contamination, 301
18.3. Monitoring for Contamination, 301
   18.3.1. Visible Microbial Contamination, 304
   18.3.2. Mycoplasma, 305
   18.3.3. Fluorescence Staining for Mycoplasma, 306
   Protocol 18.2. Fluorescence Detection of Mycoplasma, 306
      18.3.4. PCR for Mycoplasma, 307
   Protocol 18.3. Detection of Mycoplasma by PCR, 307
      18.3.5. Alternative Methods for Detecting Mycoplasma, 310
      18.3.6. Mycoplasma Detection Services, 311
      18.3.7. Viral Contamination, 311
20. Quantitation, 335
20.1. Cell Counting, 335
   20.1.1. Hemocytometer, 335
Protocol 20.1. Cell Counting by Hemocytometer, 335
   20.1.2. Electronic Counting, 339
Protocol 20.2. Electronic Cell Counting by Electrical Resistance, 340
   20.1.3. Stained Monolayers, 342
   20.1.4. Flow Cytometry, 343
20.2. Cell Weight, 344
20.3. DNA Content, 344
   Protocol 20.3. DNA Estimation by Hoechst 33258, 345
20.4. Protein, 345
   20.4.1. Solubilization of Sample, 345
   20.4.2. Bradford Assay, 345
Protocol 20.4. Protein Estimation by the Bradford Method, 345
20.5. Rates of Synthesis, 346
   20.5.1. DNA Synthesis, 346
   Protocol 20.5. Estimation of DNA Synthesis by [3H]Thymidine Incorporation, 346
   20.5.2. Protein Synthesis, 347
Protocol 20.6. Protein Synthesis, 347
20.6. Preparation of Samples for Enzyme Assay and Immunoassay, 348
20.7. Cytometry, 348
   20.7.1. In situ Labeling, 348
   20.7.2. Flow Cytometry, 348
20.8. Replicate Sampling, 348
   20.8.1. Data Acquisition, 349
   20.8.2. Data Analysis, 349
20.9. Cell Proliferation, 349
   20.9.1. Experimental Design, 349
   20.9.2. Growth Cycle, 350
Protocol 20.7. Growth Curve with a Monolayer in Flasks, 351
Protocol 20.8. Growth Curve with a Monolayer in Multiwell Plates, 352
   20.9.3. Analysis of Monolayer Growth Curves, 353
   20.9.4. Medium Volume, Cell Concentration, and Cell Density, 353
   20.9.5. Suspension Cultures, 355
Protocol 20.9. Growth Curve with Cells in Suspension, 355
   20.9.6. Phases of the Growth Cycle, 355
   20.9.7. Derivatives from the Growth Curve, 357
20.10. Plating Efficiency, 357
   Protocol 20.10. Determination of Plating Efficiency, 358
   20.10.1. Analysis of Colony Formation, 359
20.11. Labeling Index, 360
   Protocol 20.11. Labeling Index with
   [³H]Thymidine, 361
   20.11.1. Growth Fraction, 361
   20.11.2. Mitotic Index, 363
   20.11.3. Division Index, 363
20.13. Cell Migration, 363

21. Cytotoxicity, 365
21.2. In vitro Limitations, 366
   21.2.1. Pharmacokinetics, 366
   21.2.2. Metabolism, 366
   21.2.3. Tissue and Systemic Responses, 366
21.3. Nature of the Assay, 366
   21.3.1. Viability, 366
   by Dye Exclusion, 367
   Protocol 21.2. Estimation of Viability
   by Dye Uptake, 367
   21.3.2. Survival, 368
   Protocol 21.3. Clonogenic Assay for Attached Cells, 368
   21.3.3. Assays Based on Cell Proliferation, 372
   21.3.4. Metabolic Cytotoxicity Assays, 372
   21.3.5. Microtiter Assays, 372
   Protocol 21.4. MTT-Based Cytotoxicity Assay, 373
   21.3.6. Comparison of Microtiter with
   Clonogenic Survival, 376
   21.3.7. Drug Interaction, 376
21.4. Applications of Cytotoxicity Assays, 377
   21.4.1. Anticancer Drug Screening, 377
   21.4.2. Predictive Drug Testing for
   Tumors, 377
   21.4.3. Testing Pharmaceuticals, 377
21.5. Genotoxicity, 377
   21.5.1. Mutagenesis Assay by Sister Chromatid
   Exchange, 377
   Protocol 21.5. Sister Chromatid Exchange, 378
   21.5.2. Carcinogenicity, 380
21.6. Inflammation, 380

22. Specialized Cells, 383
22.1. Cell Culture of Specialized Cells, 385
22.2. Epithelial Cells, 385
   22.2.1. Epidermis, 385
   Protocol 22.1. Epidermal Keratinocytes, 387
   22.2.2. Cornea, 390
   Protocol 22.2. Corneal Epithelial Cells, 390
   22.2.3. Breast, 391
   Protocol 22.3. Preparation of Mammary Epithelial Cells
   from Reduction Mammaplasty Specimens, 392
   22.2.4. Cervix, 393
   Protocol 22.4. Cervical Epithelium, 393
   22.2.5. Gastrointestinal Tract, 395
   Protocol 22.5. Isolation and Culture of Colonic
   Crypts, 395
   22.2.6. Liver, 397
   22.2.7. Hepatocyte Primary Cultures, 397
   Protocol 22.6A. Isolation of Rat Hepatocytes, 397
   22.2.8. HepaRG Human Hepatocytes, 399
   Protocol 22.6B. Purification of HepaRG Human
   Hepatocytes, 399
   22.2.9. Pancreas, 401
   Protocol 22.7. Pancreatic Epithelium, 401
   22.2.10. Kidney, 402
   Protocol 22.8. Kidney Epithelium, 403
   22.2.11. Bronchial and Tracheal
   Epithelium, 404
   Protocol 22.9. Bronchial and Tracheal Epithelium, 404
   22.2.12. Oral Epithelium, 405
   Protocol 22.10. Oral Keratinocytes, 405
   22.2.13. Prostate, 406
   Protocol 22.11. Prostatic Epithelium, 407
22.3. Mesenchymal Cells, 408
   22.3.1. Connective Tissue, 408
   22.3.2. Adipose Tissue, 408
   Protocol 22.12. Primary Culture of Adipose
   Cells, 409
   22.3.3. Muscle, 410
   Protocol 22.13. Isolation and Culture of Smooth Muscle
   Cells, 410
   Protocol 22.14. Culture of Myoblasts from Adult Skeletal
   Muscle, 411
   Protocol 22.15. Single Myofiber Culture from
   Skeletal Muscle, 413
   22.3.4. Cartilage, 414
   Protocol 22.16. Chondrocytes in Alginate Beads, 414
   22.3.5. Bone, 416
   Protocol 22.17. Osteoblasts, 417
   22.3.6. Endothelium, 418
   Protocol 22.18. Isolation and Culture of Vascular
   Endothelial Cells, 419
22.4. Neuroectodermal Cells, 422
   22.4.1. Neurons, 422
   Protocol 22.19. Cerebellar Granule Cells, 422
   22.4.2. Glial Cells, 423
   Protocol 22.20. Primary Culture of Human
   Astrocytes, 424
   Protocol 22.21. Olfactory Ensheathing Cells, 426
   22.4.3. Endocrine Cells, 428
   Protocol 22.22. Culture of Melanocytes, 429
22.5. Hematopoietic Cells, 430
22.6. Gonads, 432
23. Stem Cells, Germ Cells, and Amniocytes, 433

23.1. Stem Cells, 433

23.1.1. Embryonic Stem Cells, 433
23.1.2. Derivation of Mouse Embryonic Stem Cells, 433

Protocol 23.1. Derivation and Primary Culture of Mouse Embryonic Stem Cells, 434

23.1.3. Subculture and Propagation of Mouse Embryonic Stem Cells, 436

Protocol 23.2. Propagation of Mouse Embryonic Stem Cell Lines, 438

23.1.4. Primary Culture of Human Embryonic Stem Cells, 439

Protocol 23.3. Derivation of Human Embryonic Stem Cells, 440

23.1.5. Passaging hES Cells, 440

Protocol 23.4. Manual Passage of hES Cells, 441

23.1.6. Pluripotent Stem Cells from Fish Embryos, 442

Protocol 23.5. Cell Cultures from Zebrafish Embryos, 443

23.2. Germ Cells, 445

23.3. Extraembryonic Cells, 445

23.3.1. Culture of Amniocytes, 445

Protocol 23.6. Culture of Amniocytes, 445

23.3.2. Cells from Neonates and Juveniles, 449

23.3.3. Multipotent Stem Cells from the Adult, 449

23.3.4. MSCs from Human Bone Marrow, 450

Protocol 23.7. MSC Production from Human Bone Marrow, 450

23.3.5. Induced Pluripotent Stem Cells, 452

Protocol 23.8. Reprogramming Human Dermal Fibroblasts for the Generation of Pluripotent Stem Cells, 453

Protocol 23.9. A. Generation of Human Dermal Fibroblast Cell Lines, 453

Protocol 23.10. B. Generation of High Titers of Infective Virus Coding for iPS Factors, 453

23.3.6. Long-Term Bone Marrow Cultures from Mouse, 455

Protocol 23.11. Long-Term Hematopoietic Cell Cultures from Mouse Bone Marrow, 456

23.3.7. Long-Term Culture of Human Primitive Hemopoietic Cells, 457


23.3.8. Hematopoietic Colony-Forming Assays, 461

24. Culture of Tumor Cells, 463

24.1. Problems of Tumor Cell Culture, 463

24.2. Sampling, 464

24.2.1. Selection of Representative Cells, 464

24.2.2. Preservation of Tissue by Freezing, 464

Protocol 24.1. Freezing Biopsies, 465

24.3. Disaggregation, 465

24.4. Primary Culture, 465

24.5. Selective Culture of Tumor Cells, 466

24.5.1. Selective Media, 466

24.5.2. Confluent Feeder Layers, 466

Protocol 24.2. Growth on Confluent Feeder Layers, 466

24.5.3. Suspension Cloning, 467

24.5.4. Xenografts, 467

24.6. Development of Cell Lines, 468

24.6.1. Subculture of Primary Tumor Cultures, 468

24.6.2. Continuous Cell Lines, 469

24.7. Characterization of Tumor Cell Cultures, 470

24.7.1. Heterogeneity of Tumor Cultures, 470

24.7.2. Histotypic Culture, 470

24.8. Specific Tumor Types, 471

24.8.1. Breast, 471

Protocol 24.3. Culture of Mammary Tumor Cells, 472

24.8.2. Lung, 472

24.8.3. Stomach, 473

24.8.4. Colon, 473

Protocol 24.4. Culture of Colorectal Tumors, 473

24.8.5. Pancreas, 475

24.8.6. Ovary, 475

24.8.7. Prostate, 476

24.8.8. Bladder, 476

24.8.9. Skin, 476

24.8.10. Cervix, 477

24.8.11. Gliona, 477


24.8.13. Seminoma, 478

24.8.14. Lymphoma and Leukemia, 478

Protocol 24.5. Establishment of Continuous Cell Lines from Leukemia/Lymphoma, 478

25. Three-Dimensional Culture, 481

25.1. Cell Interaction and Phenotypic Expression, 481

25.1.1. Effect of Cell Density, 481
25.1.2. Reciprocal Interactions, 481
25.1.3. Choice of Models, 482

25.2. Organ Culture, 482
25.2.1. Gas and Nutrient Exchange, 482
25.2.2. Structural Integrity, 484
25.2.3. Growth and Differentiation, 484
25.2.4. Limitations of Organ Culture, 484
25.2.5. Types of Organ Culture, 484
Protocol 25.1. Organ Culture, 485

25.3. Histotypic Culture, 486
25.3.1. Gel and Sponge Techniques, 486
25.3.2. Hollow Fibers, 487
25.3.3. Spheroids, 487
Protocol 25.2. 3-D Culture in Spheroids, 488
25.3.4. Rotating Chamber Systems, 489
25.3.5. Immobilization of Living Cells in Alginate, 490
25.3.6. Filter Well Inserts, 490
Protocol 25.3. Filter Well Inserts, 491
25.3.7. Cultures of Neuronal Aggregates, 492
Protocol 25.4. Neuronal Aggregates, 492

25.4. Organotypic Culture, 493
25.4.1. Tissue Equivalents, 494
25.4.2. Tissue Engineering, 495

25.5. Imaging Cells in 3-D Constructs, 495

26. Scale-up and Automation, 497
26.1. Scale-up in Suspension, 497
Protocol 26.1. Stirred 4-Liter Batch
Suspension Culture, 498
26.1.1. Continuous Culture, 500
26.1.2. Scale and Complexity, 500
26.1.3. Mixing and Aeration, 501

26.2. Scale-up in Monolayer, 503
26.2.1. Multisurface Propagators, 504
Protocol 26.2. NUNC Cell Factory, 504
26.2.2. Roller Culture, 505
Protocol 26.3. Roller Bottle Culture, 505
26.2.3. Microcarriers, 506
Protocol 26.4. Microcarriers, 508
26.2.4. Large Microcarriers, 509
26.2.5. Perfused Monolayer Culture, 509

26.3. Process Control, 510
26.4. Automation, 513
26.4.1. Robotic Cell Culture, 513
26.4.2. High-Throughput Screening, 514

27. Specialized Techniques, 517
27.1. Lymphocyte Preparation, 517
27.1.1. Isolation by Density, 517
Protocol 27.1. Preparation of Lymphocytes, 517
27.1.2. Blast Transformation, 518
Protocol 27.2. PHA Stimulation of Lymphocytes, 518

27.2. Autoradiography, 518
Protocol 27.3. Microautoradiography, 519

27.3. Time-Lapse Recording, 522
Protocol 27.4. Time-Lapse Video Recording, 523

27.4. Cell Synchrony, 525
27.4.1. Cell Separation, 525
27.4.2. Blockade, 525

27.5. Culture of Cells from Poikilothersms, 525
27.5.1. Fish Cells, 525
27.5.2. Insect Cells, 526
Protocol 27.5. Propagation of Insect Cells, 526

27.6. Somatic Cell Fusion, 527
27.6.1. Cell Hybridization, 527
Protocol 27.6. Cell Hybridization, 527
27.6.2. Nuclear Transfer, 529

27.7. Production of Monoclonal Antibodies, 529
Protocol 27.7. Production of Monoclonal Antibodies, 529

28. Training Programs, 533

28.1. Objectives, 533

28.2. Preparative and Manipulative Skills, 533
Exercise 1 Sterile Pipetting and Transfer of Fluids, 536
Exercise 2 Washing and Sterilizing Glassware, 538
Exercise 3 Preparation and Sterilization of Water, 538
Exercise 4 Preparation and Sterilization of Dulbecco’s Phosphate-Buffered Saline (D-PBS) without Ca²⁺ and Mg²⁺ (D-PBSA), 539
Exercise 5 Preparation of pH Standards, 540
Exercise 6 Preparation of Stock Medium from Powder and Sterilization by Filtration, 541

28.3. Basic Cell Culture Techniques, 543
Exercise 7 Observation of Cultured Cells, 543
Exercise 8 Preparing Sterile Medium for Use, 545
Exercise 9 Feeding a Monolayer Culture, 546
Exercise 10 Preparation of Complete Medium from 10× Stock, 547
Exercise 11 Counting Cells by Hemocytometer and Electronic Counter, 548
Exercise 12 Subculture of Cells Growing in Suspension, 551
Exercise 13 Subculture of Cell Lines Growing in Monolayer, 552
Exercise 14 Staining a Monolayer Cell Culture with Giemsa, 554
Exercise 15 Construction and Analysis of Growth Curve, 556

28.4. Advanced Exercises, 557
Exercise 16 Cell Line Characterization, 558
Exercise 17 Detection of Mycoplasma, 559
Exercise 18 Cryopreservation of Cultured Cells, 560
Exercise 19 Primary Culture, 563
Exercise 20 Cloning of Monolayer Cells, 566

28.5. Specialized Exercises, 568