

Contents

Preface	v
List of protocols	xiii
Abbreviations	xvii

1 Introduction to the principles of flow cytometry 1

Nigel P. Carter and Michael G. Ormerod

1	Introduction	1
2	Techniques for sample preparation	2
3	Fluidics	3
4	Detection and measurement	7
5	Cell sorting	21
	References	22

2 Fluorescence and fluorochromes 23

Michael G. Ormerod

1	Introduction	23
2	Principles	23
3	Fluorophores used to label covalently other probes	25
4	Fluorophores used to label directly cell components	28
	References	33

3 Preparing suspensions of single cells 35

Michael G. Ormerod

1	Introduction	35
2	Cells from peripheral blood	35
3	Leucocytes from other tissue	38
4	Other types of cell	39
5	Cultured cells	44
	References	45

4 Flow sorting 47*Nigel P. Carter and Michael G. Ormerod*

- 1 Introduction 47
- 2 Principles of flow sorting 47
- 3 Parameters which affect sorting 50
- 4 Practical considerations 52
- 5 Specialized features 57
- 6 Other flow-sorting systems 58
 - Further reading 59
 - References 59

5 Immunofluorescence of surface markers 61*Michael R. Loken, Cherie L. Green and Denise A. Wells*

- 1 Introduction 61
- 2 Instrument standardization 63
- 3 Testing sensitivity and resolution 68
- 4 Immunofluorescence 70
- 5 Staining for surface markers 70
- 6 Titration of antibodies 72
- 7 CD nomenclature 72
- 8 Population assignment 72
- 9 Constructing an immunophenotyping panel 73
- 10 Isotype controls 80
- 11 DNA content/surface marker analysis 81
 - References 82

6 Analysis of DNA—general methods 83*Michael G. Ormerod*

- 1 Introduction 83
- 2 Definitions 83
- 3 General comments 85
- 4 Experimental methods 89
- 5 Deconvolution of the DNA histogram 93
 - Acknowledgement 96
 - References 96

7 Further clinical applications 99*Terry Hoy, Steve Garner, Brian K. Shenton, Alison E. Bell, Mark W. Lowdell, John Farrant, Margaret North, and Carrock Sewell*

- 1 Introduction 99
- 2 The enumeration of reticulocytes in peripheral blood 99

- 3 The enumeration of CD34+ cells in peripheral blood stem-cell harvests and bone-marrow harvests 101
- 4 Simultaneous detection of granulocyte- and lymphocyte-reactive antibodies 104
- 5 Detection of platelet-reactive antibodies 107
- 6 Application of crossmatching by flow cytometry to transplantation 109
- 7 Monitoring anti-thymocyte globulin (ATG)/anti-lymphocyte globulin (ALG) therapy in transplantation 111
- 8 Measurement of cell-mediated cytotoxicity by flow cytometry 114
- 9 Monitoring cell activation in response to antigens or mitogens 116
- 10 Measuring intracellular cytokines by flow cytometry 117
- References 122

8 Quality assurance in the clinical laboratory 125

Jan W. Gratama and David Barnett

- 1 Introduction 125
- 2 Reference materials for calibration 125
- 3 Internal quality-control procedures 127
- 4 External quality assurance 130
- 5 Conclusions 131
- References 132

9 Measurement of cytoplasmic and nuclear antigens 133

Jørgen K. Larsen

- 1 Introduction 133
- 2 Methods of cell permeabilization 134
- 3 Methods of cell staining and analysis 142
- 4 Examples of intracellular antigens that can be measured by flow cytometry 146
- References 155

10 Analysis of DNA—measurement of cell kinetics by the bromodeoxyuridine/anti-bromodeoxyuridine method 159

George D. Wilson

- 1 Introduction 159
- 2 Basic cell-kinetic concepts 160
- 3 Background to the BrdUrd technique 162
- 4 Methods of BrdUrd incorporation 164
- 5 Tissue preparation 166
- 6 Staining procedures 167
- 7 Flow cytometry 172

--contents p.8-- --protocols p.13--

8	Examples of data and analysis	172
9	Conclusions	176
	Acknowledgement	177
	References	177

11 Analysis of cell proliferation using the bromodeoxyuridine/Hoechst-ethidium bromide method 179

Michael G. Ormerod and Martin Poot

1	Introduction	179
2	Cell culture and BrdUrd labelling	179
3	Cell staining and flow cytometry	180
4	Synchronized cells	182
5	Asynchronous cells	183
6	Troubleshooting	186
	Acknowledgement	188
	References	188

12 Chromosome analysis and sorting by flow cytometry 189

Derek C. Davies, Simon P. Monard, and Bryan D. Young

1	Introduction	189
2	Preparation of chromosome suspensions	192
3	Instrumentation	196
4	Flow sorting chromosomes for library construction	197
5	Generation of chromosome paints	198
	References	201

13 Intracellular ionized calcium, magnesium, membrane potential, and pH 203

Peter S. Rabinovitch and Carl H. June

1	Intracellular ionized calcium	203
2	Magnesium	222
3	Membrane potential	222
4	Measurement of cytoplasmic pH	227
	Acknowledgement	232
	References	232

14 Flow cytometry in the study of apoptosis 235

Michael G. Ormerod

1	Introduction	235
2	Morphological and biochemical changes	235

- 3 Measurement of DNA degradation 236
- 4 Mitochondrial membrane potential 240
- 5 Measurements of changes in the plasma membrane 243
- 6 Other measurements 245
- 7 Quantification of apoptotic cells 246
- Acknowledgement 247
- References 247

15 Further applications to cell biology 249

Michael G. Ormerod

- 1 Introduction 249
- 2 Estimating cell viability 249
- 3 Monitoring electroporation of cells 251
- 4 Measurement of oxidative burst 253
- 5 Characterizing multi-drug resistance (MDR) in cancer cells 254

A1 Safety procedures 259

- 1 Bench-top flow cytometers 259
- 2 Cell sorters 259
- 3 Chemicals 260

A2 Flow cytometers and software 261

- 1 Commercial flow cytometers 261
- 2 Software for data manipulation 263

A3 List of suppliers 265

Index 273

--contents p.8-- --protocols p.13--