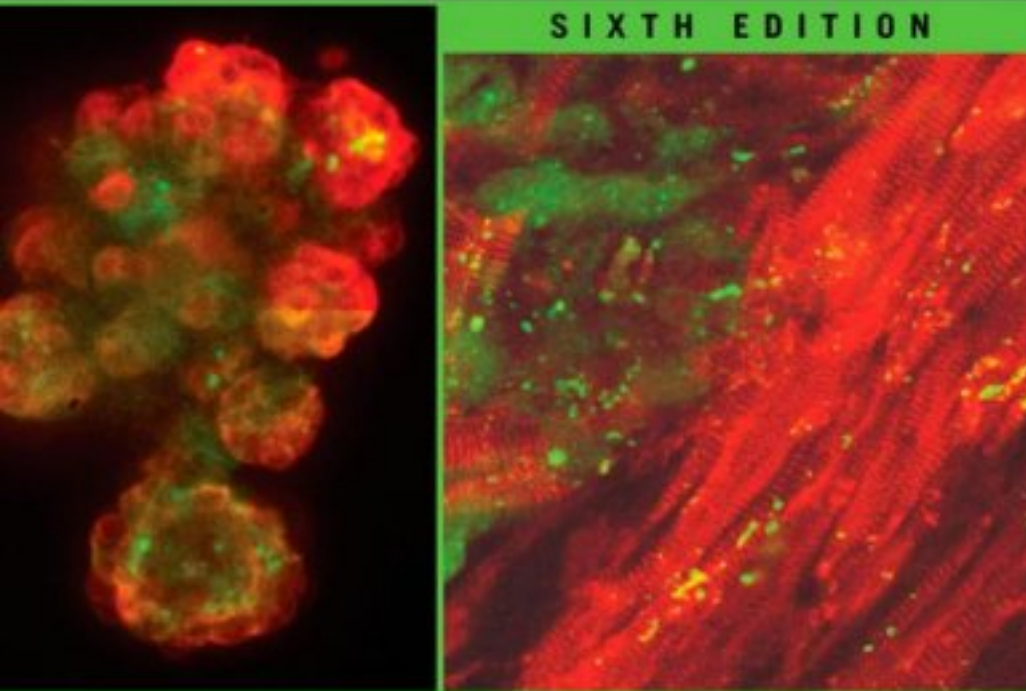



# Culture of Animal Cells

SIXTH EDITION



*A Manual of Basic Technique and Specialized Applications*

R. IAN FRESHNEY

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# CULTURE OF ANIMAL CELLS

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**A MANUAL OF BASIC TECHNIQUE  
AND SPECIALIZED APPLICATIONS**  
Sixth Edition

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Cancer Research UK Centre for Oncology and Applied Pharmacology  
Division of Cancer Sciences and Molecular Pharmacology  
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Front cover photographs: Terminal ductal lobular-like unit cultured from normal human mammary epithelium [Labarge et al., 2007] and tissue-engineered rat heart tissue after implantation [Eschenhagen & Zimmerman, 2006]. Spine: Embryoid bodies from human ES cells [Cooke & Minger, 2007]. *Rear cover*: Nestin expression in replated neurospheres from human ES cells [Jackson et al., 2007].

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This book is dedicated to all of the many friends and colleagues whose help and advice over the years has enabled me to extend the scope of this book beyond my own limited experience.

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#### Companion Website

A companion resources site for this book is available at:  
[www.wiley.com/go/freshney/cellculture](http://www.wiley.com/go/freshney/cellculture)



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# Preface and Acknowledgements

When the first edition of this book was published in 1983, although cell culture was an established technique it was still largely a research tool with a relatively small following. There was still an element of distrust that cell culture could deliver information relevant to processes *in vivo*. Largely because of the requirements of molecular genetics and virology the use of cell culture expanded into a major industrial process for the generation of biopharmaceuticals. Now the field is expanding further and entering other exciting areas of stem cell research and regenerative medicine. Perhaps one of the most exciting aspects of current progress in the field is that we can now grasp the “holy grail” of working with fully functional specialized cells in culture. A combination of selective culture conditions and manipulation of gene expression has meant that not only can we isolate and culture specialized cells, we can buy them “off the shelf,” and we can evoke a plasticity in gene expression in both primitive stem cells and mature cells previously thought to be committed to their fate.

This book is the sixth edition of *Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications*. Those who have used the previous edition will notice the extended title as some of the topics dealt with cannot be regarded as basic techniques. The book also has acquired a new chapter on stem cells, reflecting the current upsurge in interest in this area. Chapter 2, Training Programs, which is designed to enhance the use of this book as a teaching manual in addition to its role as a reference text, is now moved to the third to last chapter, on the assumption that instructors and trainees or students should have spent some time on the earlier chapters first, before attempting the exercises.

The number of color plate pages has been extended and, in combination with Figure 16.2, the book now provides

photographs of around 40 different cell lines, including primary cultures, equipment, and processes. There are four new plates, two of stem cells and two of specialized cells (Courtesy of Cell Applications, Inc.). I am greatly indebted to Yvonne Reid and Greg Sykes of ATCC, Peter Thraves of ECACC, and many others for kindly providing illustrations. I hope that the color plates, in particular, will encourage readers to look at their cells more carefully and become sensitive to any changes that occur during routine maintenance.

For most of the book, I have retained the emphasis of previous editions and focused on basic techniques with some examples of more specialized cultures and methods. These techniques are presented as detailed step-by-step protocols that should give sufficient information to carry out a procedure without recourse to the prime literature. There is also introductory material to each protocol explaining the background and supplementary information providing alternative procedures and applications. Some basic biology is explained in Chapter 2, but it is assumed that the reader will have a basic knowledge of anatomy, histology, biochemistry, and cell and molecular biology. The book is targeted at those with little or no previous experience in tissue culture, including technicians in training, senior undergraduates, graduate students, postdoctoral workers, and clinicians with an interest in laboratory science. Those working in the biotechnology industry, including cell production, screening assays, and quality assurance, should also find this book of value.

The specialized techniques chapter 27, no longer contains protocols in molecular techniques as there are many other sources of these [e.g., Sambrook and Russell, 2006; Ausubel et al., 2009], and it is also an area in which I am not

well versed. Similarly Chapter 26 on scale-up serves as an interface with biotechnology and provides some background on systems for increasing cell yield, but takes no account of full-scale biopharmaceutical production and downstream processes. The section on automation has been extended with more examples of the use of robotics in cell culture.

Protocols are given a distinct appearance from the rest of the text. Reagents that are specific to a particular protocol are detailed in the materials sections of the protocols and the recipes for the common reagents, such as Hanks's BSS or trypsin, are given in Appendix I at the end of the book. Details of the sources of equipment and materials are given in Appendix II. The suppliers' list (Appendix III) has been updated, but addresses, telephone and fax numbers, and email addresses are not provided, and only the website is given, on the assumption that all necessary contact information will be found there. Suppliers are not cited in the text unless for a specialized item.

Abbreviations used in the text are listed separately after this preface. Conventions employed throughout are D-PBSA for Dulbecco's PBS without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  and UPW for ultrapure water, regardless of how it is prepared. Concentrations are given in molarity wherever possible, and actual weights have been omitted from the media tables on the assumption that very few people will attempt to make up their own media but will, more likely, want to compare constituents, for which molar equivalents are more useful.

As always, I owe a great debt of gratitude to the authors who have contributed protocols, and to others who have advised me in areas where my knowledge is imperfect, including Robert Auerbach, Bob Brown, Mike Butler, Kenneth Calman, Roland Grafström, Richard Ham, Rob Hay, Stan Kaye, Nicol Keith, John Masters, Wally McKeehan, Rona McKie, Stephen Merry, Jane Plumb, Peter Vaughan, Paul Workman, the late John Paul, and members of the staff at ECACC, including Isobel Atkins, Jim Collins, David Lewis, Chris Morris, and Peter Thraves. I am fortunate in having had the clinical collaboration of

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I have been fortunate to receive excellent advice and support from the editorial staff of John Wiley & Sons. I would also like to acknowledge, with sincere gratitude, all those who have taken the trouble to write to me or to John Wiley & Sons with advice and constructive criticism on previous editions. It is pleasant and satisfying to hear from those who have found this book beneficial, but even more important to hear from those who have found deficiencies, which I can then attempt to rectify. I can only hope that those of you who use this book retain the same excitement that I feel about the future prospects emerging in the field.

I would like to thank my daughter Gillian and son Norman for all the help they gave me in the preparation of the first edition, many years ago, and for their continued advice and support. Above all, I would like to thank my wife, Mary, for her hours of help in compilation, proofreading, and many other tasks; without her help and support, the original text would never have been written and I would never have attained the necessary level of technical accuracy that is the keynote of a good tissue culture manual.

*R. Ian Freshney*



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