T Cell Protocols
Preface

This book is a collection of protocols, to provide novel techniques for the study of the biology of T lymphocytes.

The methods described in this book do not cover all of the techniques currently used to study T cell-mediated immune responses for the simple reason that T cell immunology is probably the immunological discipline which can be investigated with the widest variety of approaches.

The choice of chapters was made taking into account two points: First, many of the techniques that have been used for some time have been upgraded during the past few years given the greater availability of a variety of products (i.e. cytokines, chemokines, monoclonal antibodies), of refined technical devices (i.e. novel cell culture and cell analysis equipments), and the development of novel instrumentation (i.e. multiparametric flow cytometers, confocal microscopes). Therefore, in several chapters “old techniques”, which remain fundamental to T cell immunology, are described in their “modern” versions.

Secondly, the technical advancement has generated the possibility to establish novel assays to investigate T cell physiology. This is reflected in the chapters which describe the protocols that allow use of these modern approaches.

The preparation of this book has required participation of several scientists, all leading experts in their respective fields. Without their enthusiastic participation, this work would not have been possible. Therefore, I thank all authors for their contributions and accept all criticism for missing parts, or information or details, for which only I am responsible.

I hope these protocols will be useful for young investigators who approach for the first time the complex field of immunology and for those more experienced scientists who look for concise and efficacious descriptions of novel methods.
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Color Plates

Color Plate 1: Multidimensional analysis of human PBMC stimulated with either IL-6 (green), IL-4 (red) or left untreated (blue). Cells were fixed and permeabilized following protocol 3.2 and stained simultaneously with antibody cocktail A. Top panels show superimposed dot plots and histograms for T-cells (CD3\(^+\)), the bottom panels show B-cells (CD20\(^+\)). In overlays the induction of specific phosphorylation events are clearly identifiable. (see discussion on p. 41)

Color Plate 2: PBMC were stimulated with indicated cytokines, fixed and permeabilized according to protocol 3.2. T-cells (CD3\(^+\)) and B-cells (CD20\(^+\)) were gated according to markers while monocytes were gated in scatter plot. Open histograms represent untreated cells, filled histograms stimulated cells. Induction of phosphorylation is clearly identifiable (filled yellow histograms). (see discussion on p. 41)

Color Plate 3: Visualization of the data generated by the FACS analysis following protocol 3.2. The columns represent the cell subsets, T-cells, B-cells, monocytes. Each row represents a cytokine stimulation stained with one of the antibody cocktails and subsequently analyzed for the indicated phosphoprotein. The color of each block represents the fold change (log\(_2\)) in MFI in the channel corresponding to the analyzed phosphorylated protein. (see discussion on p. 42)