

# METHODS IN MOLECULAR BIOLOGY™

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# Cytokine Protocols

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 **Humana Press**

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ISSN 1064-3745 e-ISSN 1940-6029  
ISBN 978-1-61779-438-4 e-ISBN 978-1-61779-439-1  
DOI 10.1007/978-1-61779-439-1  
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2011940833

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Printed on acid-free paper

Humana Press is part of Springer Science+Business Media ([www.springer.com](http://www.springer.com))

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## Preface

Seven years have passed since the first volume of “Cytokine Protocols” was published in the series of “Methods in Molecular Biology” (Volume 249, 2004) of Humana Press. Since then, not only the number of known/characterized cytokines has drastically increased (e.g., interleukins up to IL-35) but also assays for gene expression have become more sensitive and sophisticated, allowing the simultaneous processing of higher numbers and/or smaller samples. In recent years we have witnessed the far-advanced miniaturization of microanalytical methods as well as the extensive development of bioinformatics and nanotechnology. Together these allow performing methods such as genomics, transcriptomics, and proteomics. At the same time a substantial reduction in sample size was achieved, allowing accurate determinations that were previously impossible. Single-cell based assays are expected to further extend this broad range of assays.

The first chapter written by my colleague professor A. Billiau is not only of historical importance but also brings a message of general importance to researchers using bioassays in general. Careful observation and interpretation of results obtained in two different (biological) assays with respect to possible differences may reveal the presence of other hitherto unknown cytokines to be discovered and further characterized.

The next three chapters deal with the quantification and characterization of cytokine-related RNAs. These range from the cytokine mRNAs themselves over cytokine-induced genes until miRNAs. Real-time quantitative PCR (RT-qPCR), now widely established as a standard molecular biological technique, yields accurate determinations of single cytokine mRNA transcript levels (Chapter 2). Simultaneous measurement of gene expression profiles after cytokine stimulation is made possible through application of DNA microarray techniques (Chapter 3). The eventual level of mature active mRNA depends on multiple regulatory factors and processes, among which miRNAs. Their accurate quantitative determination (as well as that of their precursors) can also be executed by RT-qPCR (Chapter 4).

The next seven chapters deal with the posttranscriptional modifications of RNA, taking place either naturally or artificially. One of the most decisive factors in determining cytokine levels and the response to it are mRNA levels, themselves being regulated by two opposite mechanisms: generation and decay, in turn regulated by cis-elements as well as trans-acting proteins. Both their characterization and evaluation yields further insight in the signal transduction processes (Chapter 5). One of the well-known mechanisms acts through the interaction of proteins with AU-rich elements in the 3'UTR of mRNAs, the involvement of which can be demonstrated using a cell-based GFP assay (Chapter 6). Although the highly selective and efficient silencing of genes by siRNAs is known already for a long time, the delivery of these siRNAs to some kinds of cells restricts its broader application. A neat way to overcome this obstacle is through their inclusion in (integrin) targeted stabilized nanoparticles (Chapter 7). Another proven method for gene silencing is through the application of carefully designed and validated hammerhead ribozymes. These can either be introduced in the cell as chemically modified ribozymes (in order to increase their half-life) or be constitutively generated in situ by appropriate plasmids (Chapter 8). A well-known and often

undesirable side effect of RNAi methodology is the induction of interferon response, either by the production of the cytokine itself or by the induction of interferon-related gene transcription. Hence, it is often difficult to distinguish between the pursued RNAi effect and the confusing interferon effects (Chapters 9 and 10). RNAi technology allows very specific targeting to a particular gene transcript and hence to a specific member in a signal transduction pathway. This very powerful approach is, however, often hampered by difficulties encountered at the introduction of the foreign DNA in the recipient cell (“hard-to-transfect cells,” e.g., primary cells) and by its possible toxicity. Therefore, different protocols and reagents should be carefully compared (Chapter 11).

The last three chapters are devoted to observations at the protein level. Following the identification of a novel cytokine biological activity, the next big challenge is the isolation, purification, and characterization of its first contact with the cell, i.e., its membrane receptor. Ligand affinity chromatography is the method of choice, allowing in most cases the isolation of sufficient amounts of intact receptor for partial sequence determination followed by full sequence prediction from data banks. Moreover, this method may also lead to the discovery of unexpected, unpredicted (non-receptor), interacting proteins (Chapter 12). Accurate and sensitive detection of cytokine levels is of prime importance in the evaluation of their biological activity, both in situ (intracellular) and in vitro (solution) methods are needed. Application of fluorescently labeled monoclonal antibodies in combination with flow cytometry on permeabilized cells allows sensitive detection even in individual cells (Chapter 13). As already explained in the first chapter, sensitive and specific detection of the biological activity of cytokines is of utmost importance. It is well known that each cytokine is quantified most specifically, accurately, and with the lowest detection limit on a different cell type, thus obliging researchers that work with different cytokines to maintain a whole series of cultures of various cells, each with their own detection system). This problem can be partly circumvented by constructing cell lines with chimeric receptors, the extracellular part of them being specific for each cytokine, the intracellular part being the same for all and thus requiring only one kind of signal detection (Chapter 14).

*Heverlee, Belgium*

*Marc De Ley*

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