

Natural Killer Cell Protocols

Cellular and Molecular Methods
Second Edition

Edited by

Kerry S. Campbell

 Humana Press

Natural Killer Cell Protocols Cellular And Molecular Methods

Ian M. Clark



Natural Killer Cell Protocols Cellular And Molecular Methods:

Natural Killer Cell Protocols Kerry S. Campbell, 2010-05-06 As the research has continued it has become increasingly clear that natural killer NK cells are critical sentinels of the innate immune response playing important roles in protecting the body from numerous pathogens and cancer in addition to contributing to normal pregnancy and impacting the outcomes of transplantation While the first edition provided a valuable collection of classical cellular and in vivo techniques to study NK cell functions the Second Edition of Natural Killer Cell Protocols Cellular and Molecular Methods brings together more recently developed methods more refined techniques and detailed protocols designed to study NK cells within specialized tissue sites in both mice and humans In this collection of methods international leaders in the field cover topics ranging from the analysis of the various stages of NK cell development and maturation to specialized techniques for the identification of ligands for NK cell receptors This volume also includes an appendix providing a rich resource summarizing available reagents to study NK cells cross referencing KIR nomenclature and detailing the many HLA ligands for various KIR family members As a volume in the highly successful Methods in Molecular Biology™ series format chapters include introductions to their respective topics lists of the necessary materials and reagents step by step readily reproducible laboratory protocols and thorough notes sections highlighting tips on troubleshooting and avoiding known pitfalls Comprehensive and cutting edge Natural Killer Cell Protocols Cellular and Molecular Methods Second Edition seeks to aid researchers and further advance our understanding of the functions maturation and regulation of these fascinating and dynamic cells

Natural Killer Cell Protocols Kerry S. Campbell, Marco Colonna, 2008-02-03 In Natural Killer Cell Protocols Cellular and Molecular Methods Kerry S Campbell and Marco Colonna have assembled a comprehensive collection of readily reproducible methods designed to study natural killer NK cells from the broadest variety of viewpoints These include not only classic techniques but also new approaches to standard methods newly evolved techniques that have become valuable for specific applications and unique models for manipulating and studying NK cells Among the advanced methods covered are those for in vitro transendothelial migration in vivo detection of cells migrating into tumors immunofluorescence staining of intracellular cytokines and in vitro NK cell development Valuable techniques for specific applications include vaccinia virus protein expression soluble KIR Fc fusions for HLA class I binding assays calcium mobilization in cell conjugates and identification of heterodimeric receptor complexes using cDNA library expression cloning No less important are accounts of such classic methods as hybrid resistance ADCC viral defense target cell cytotoxicity assays cloning and culturing tumor immunotherapy and generation of HLA class I transfected target cells Natural Killer Cell Protocols Cellular and Molecular Methods offers immunologists cancer researchers virologists and cell biologists today s most comprehensive collection of both established and cutting edge techniques methods that will contribute significantly to advancing our understanding of this fascinating and critically important class of cells

Cytoskeleton Methods and Protocols Ray H. Gavin, 2008-02-03 Over the past two

decades experimental studies have solidified the interpretation of the cytoskeleton as a highly dynamic network of microtubules actin microfilaments intermediate filaments and myosin filaments Rather than a network of disparate fibers these polymers are often interconnected and display synergy which is the combined action of two or more cytoskeletal polymers to achieve a specific cellular structure or function Cross communication among cytoskeletal polymers is thought to be achieved through cytoskeletal polymer accessory proteins and molecular motors that bind two or more cytoskeletal polymers Development of the modern concept of the cytoskeleton is a direct result of growth of advances in experimental tools and reagents that are available to cell and molecular biologists Technological advances and refinements in cell imaging have made it possible to selectively image a single cytoskeletal polymer and monitor its dynamics through the use of fluorescence probes in vitro and in vivo Two decades ago cytoskeletal research was limited to a few perturbation reagents that included colchicine and cytochalasin Today the perturbation arsenal has expanded to a highly selective group of reagents that includes Taxol nocodazole benomyl latrunculin jasplakinolide and such endogenous proteins as gelsolin These reagents enable the investigator to selectively perturb or destroy a cytoskeletal polymer while leaving other cytoskeletal polymers intact Site specific monoclonal antibodies that target a specific cytoskeletal polymer have proven to be highly selective affinity tools for cytoskeletal research

Immunotoxin Methods and Protocols Walter A. Hall, 2008-02-02 Immunotoxins represent a new class of human therapeutics that have widespread applications and a potential that has not yet been fully recognized since they were first conceived of by Paul Ehrlich in 1906 The majority of advances in the development and implementation of immunotoxins has occurred over the last 20 years The reasons for this use of immunotoxins in basic science and clinical research are the powerful concurrent advances in genetic engineering and receptor physiology Recombinant technology has allowed investigators to produce sufficient quantities of a homogeneous compound that allows clinical trials to be performed The identification of specific receptors on malignant cell types has enabled scientists to generate immunotoxins that have had positive results in clinical trials As more cellular targets are identified in coming years additional trials will be conducted in different disease states affecting still larger patient populations Modulation of the immune system to decrease the humoral response to immunotoxins may improve their overall efficacy As increasingly more effective compounds are generated it will be necessary to decrease the local and systemic toxicity associated with these agents and methods for doing so are presently being developed The work presented in Immunotoxin Methods and Protocols focuses on three specific areas of immunotoxin investigation that are being conducted by experts throughout the world The first section describes the construction and development of a variety of immunotoxins

Complement Methods and Protocols B. Paul Morgan, 2008-02-05 The complement system first described more than a century ago was for many years the ugly duckling of the immunology world but no more Complement in recent years has blossomed into a fascinating and fast moving field of immediate relevance to clinical scientists in fields as diverse as transplantation biology virology and inflammation Despite its

emergence from the shadows complement retains an unwarranted reputation for being difficult This impression derives in large part from the superficially complicated nomenclature a relic of the long and tortuous process of unraveling the system of naming components in order of discovery rather than in a systematic manner Once the barrier of nomenclature has been surmounted then the true simplicity of the system becomes apparent Complement comprises an activation system and a cytolytic system The former has diverged to focus on complement to distinct targets bacteria immune complexes and others so that texts now describe three activation pathways closely related to one another but each with some unique features The cytolytic pathway is the same regardless of the activation process and kills cells by creating pores in the membrane Complement plays an important role in killing bacteria and is essential for the proper handling of immune complexes Problems occur when complement is activated in an inappropriate manner the potent inflammation inducing products of the cascade then cause unwanted tissue damage and destruction

Bioinformatics Methods and Protocols Stephen Misener, Stephen A. Krawetz, 2008-02-02 Computers have become an essential component of modern biology They help to manage the vast and increasing amount of biological data and continue to play an integral role in the discovery of new biological relationships This in silico approach to biology has helped to reshape the modern biological sciences With the biological revolution now among us it is imperative that each scientist develop and hone today's bioinformatics skills if only at a rudimentary level *Bioinformatics Methods and Protocols* was conceived as part of the *Methods in Molecular Biology* series to meet this challenge and to provide the experienced user with useful tips and an up to date overview of current developments It builds upon the foundation that was provided in the two volume set published in 1994 entitled *Computer Analysis of Sequence Data* We divided *Bioinformatics Methods and Protocols* into five parts including a thorough survey of the basic sequence analysis software packages that are available at most institutions as well as the design and implementation of an essential introductory *Bioinformatics* course In addition we included sections describing specialized noncommercial software databases and other resources available as part of the World Wide Web and a stimulating discussion of some of the computational challenges biologists now face and likely future solutions

Connexin Methods and Protocols Roberto Bruzzone, Christian Giaume, 2008-02-05 Direct cell-cell communication is a common property of multicellular organisms that is achieved through membrane channels which are organized in gap junctions The protein subunits of these intercellular channels the connexins form a multigene family that has been investigated in great detail in recent years It has now become clear that in different tissues connexins speak several languages that control specific cellular functions This progress has been made possible by the availability of new molecular tools and the improvement of basic techniques for the study of membrane channels as well as by the use of genetic approaches to study protein function in vivo More important connexins have gained visibility because mutations in some connexin genes have been found to be linked to human genetic disorders *Connexin Methods and Protocols* presents in detail a collection of techniques currently used to study the cellular and molecular

biology of connexins and their physiological properties The field of gap junctions and connexin research has always been characterized by a multidisciplinary approach combining morphology biochemistry biophysics and cellular and molecular biology This book provides a series of cutting edge protocols and includes a large spectrum of practical methods that are available to investigate the function of connexin channels Connexin Methods and Protocols is divided into three main parts

Molecular Methods in Developmental Biology Matt Guille, 2008-02-03 The process whereby a single cell the fertilized egg develops into an adult has fascinated for centuries Great progress in understanding that process however has been made in the last two decades when the techniques of molecular biology have become available to developmental biologists By applying these techniques the exact nature of many of the interactions responsible for forming the body pattern are now being revealed in detail Such studies are a large and it seems ever expanding part of most life science groups It is at newcomers to this field that this book is primarily aimed A number of different plants and animals serve as common model organisms for developmental studies In Molecular Methods in Developmental Biology Xenopus and Zebrafish a range of the molecular methods applicable to two of these organisms are described these are the South African clawed frog *Xenopus laevis* and the zebrafish *Brachydanio rerio* The embryos of both of these species develop rapidly and externally making them particularly suited to investigations of early vertebrate development However both *Xenopus* and zebrafish have their own advantages and disadvantages *Xenopus* have large robust embryos that can be manipulated surgically with ease but their pseudotetraploidy and long generation time make them unsuitable candidates for genetics This disadvantage may soon be overcome by using the diploid *Xenopus tropicalis* and early experiments are already underway The transparent embryos of zebrafish render them well suited for in situ hybridization and immunohistochemistry and good for observing mutations in genetic screens

Neurotrophin Protocols Robert A. Rush, 2008-02-03 The past decade has seen an extraordinary growth in research interest in neurotrophic factors and the study of the neurotrophin family has led this activity Nevertheless this area of research has often struggled as a result of techniques that were either inadequate or just emerging from other research fields and disciplines Neurotrophin Protocols has brought together many leaders in the neurotrophin field who detail their special expertise in a wide variety of techniques Though most procedures are valid across many different fields of research some of those described here have been developed to address particular issues within the neurotrophic factor field The protocols cover a broad range of biochemical histological and biological techniques that are often required by the modern laboratory However all have been written with sufficient detail to allow any laboratory to achieve proficiency without need of reference to other texts Neurotrophin Protocols is divided into four sections dealing with protein RNA recombinant and in vivo techniques Protein techniques have in general been less successfully employed than those dealing with RNA or DNA However procedures that achieve localization and quantification of the neurotrophins are now being used more extensively Their inclusion here should assist further studies at the protein level Transgenic cell lines and animals are commonplace in

the scientific research literature but their inclusion in several chapters in this book provide some novel uses that are not readily available elsewhere

DNA Topoisomerase Protocols Neil Osheroff, Mary-Ann Bjornsti, 2008-02-05 Beginning with the Escherichia coli protein or bacterial DNA topoisomerase I an ever increasing number of enzymes have been identified that catalyze changes in the linkage of DNA strands DNA topoisomerases are ubiquitous in nature and have been shown to play critical roles in most processes involving DNA including DNA replication transcription and recombination These enzymes further constitute the cellular targets of a number of clinically important antibacterial and anticancer agents Thus further studies of DNA topology and DNA topoisomerases are critical to advance our understanding of the basic biological processes required for cell cycle progression cell division genomic stability and development In addition these studies will continue to provide critical insights into the cytotoxic action of drugs that target DNA topoisomerases Such mechanistic studies have already played an important role in the development and clinical application of antimicrobial and chemotherapeutic agents The two volumes of DNA Topoisomerase Protocols are designed to help new and established researchers investigate all aspects of DNA topology and the function of these enzymes The chapters are written by prominent investigators in the field and provide detailed background information and step by step experimental protocols The topics covered in Part I DNA Topology and Enzymes range from detailed methods to analyze various aspects of DNA structure from linking number knotting unknotting site specific recombination and decatenation to the overexpression and purification of bacterial and eukaryotic DNA topoisomerases from a variety of cell systems and tissues

Amino Acid Analysis Protocols Catherine Cooper, 2008-02-05 A collection of classic and cutting edge techniques of high utility in answering specific biological questions about amino acids Common methods include those based on HPLC or gas chromatography separation and analysis after precolumn derivatization New techniques based on capillary electrophoresis separation high performance anion exchange chromatography and mass spectrometry are also presented Each method is described in step by step detail to ensure successful experimental results and emphasizes sample preparation particularly the collection and storage of bodily fluids Up to date and highly practical Amino Acid Analysis Protocols offers analytical and clinical chemists as well as a broad range of biological and biomedical investigators a rich compendium of laboratory tools for the productive analysis of both common and uncommon amino acids

Chaperonin Protocols Christine Schneider, 2008-02-05

Bacterial Toxins Otto Holst, 2008-02-05 The interest of investigators across a broad spectrum of scientific disciplines has been steadily stimulated by the field of bacterial toxin research an area that makes use of a large variety of biological chemical physicochemical and medically oriented approaches Researchers studying bacterial toxins need to be acquainted with all these disciplines in order to work effectively in the field To date there has been no published collection offering detailed descriptions of the techniques and methods needed by researchers operating across the field diverse areas The present volume Bacterial Toxins Methods and Protocols is intended to fill this gap Bacterial Toxins Methods and Protocols consists of two sections one on protein toxins

15 chapters and one on endotoxins 5 chapters Each section is introduced by an overview article Chapters 1 and 16 The protocols collected represent state of the art techniques that each have high impact on future bacterial toxin research All methods are described by authors who have regularly been using the protocol in their own laboratories Included in each chapter is a brief introduction to the method being described

Nuclease Methods and Protocols Catherine H. Schein, 2008-02-03 Nucleases enzymes that restructure or degrade nucleic acid polymers are vital to the control of every area of metabolism They range from housekeeping enzymes with broad substrate ranges to extremely specific tools 1 Many types of nucleases are used in lab protocols and their commercial and clinical uses are expanding The purpose of Nuclease Methods and Protocols is to introduce the reader to some we characterized protein nucleases and the methods used to determine their activity structure interaction with other molecules and physiological role Each chapter begins with a mini review on a specific nuclease or a nuclease related theme Although many chapters cover several topics they were arbitrarily divided into five parts Part I Characterizing Nuclease Activity includes protocols and assays to determine general processive distributive or specific mechanisms Methods to assay nuclease products identify cloned nucleases and determine their physiological role are also included here Part II Inhibitors and Activators of Nucleases summarizes assays for measuring the effects of other proteins and small molecules Many of these inhibitors have clinical relevance Part III Relating Nuclease Structure and Function provides an overview of methods to determine or model the 3 D structure of nucleases and their complexes with substrates and inhibitors A 3 D structure can greatly aid the rational design of nucleases and inhibitors for specific purposes Part IV Nucleases in the Clinic summarizes assays and protocols suitable for use with tissues and for nuclease based therapeutics

Mycotoxin Protocols Mary W. Trucksess, Albert E. Pohland, 2008-02-05 Mycotoxins produced by molds are common contaminants of many important crops including wheat corn rice and peanuts Some mycotoxins are found in fruits and vegetables These contaminants have a broad range of toxic effects including carcinogenicity neurotoxicity and reproductive and developmental toxicity The occurrence of mycotoxins in foods is an unavoidable worldwide problem About 80 countries have imposed regulatory limits to minimize human and animal exposure to mycotoxins Regulatory limits including international standards have tremendous economic impact and must be developed using science based risk assessments The purpose of Mycotoxin Protocols is to provide the scientific and technological basis for analytical methods for use in obtaining the exposure data needed for risk assessments Mycotoxin Protocols is divided into four sections which are interconnected The first section Chapters 1 5 describe the general techniques for mycotoxin analysis with emphasis on the importance of method validation based on statistical parameters sampling procedures for collecting a sample as representative as possible of a bulk lot the isolation of mycotoxins for use as analytical standards or for toxicological studies the evaluation of purity and preparation of standards and the detection and identification of impurities in isolated mycotoxins Sections 2 4 Chapters 6 19 describe the most current chromatographic and immunochemical methods for studies on the

major mycotoxins *Mass Spectrometry of Proteins and Peptides* John R. Chapman, 2008-02-05 Little more than three years down the line and I am already writing the Preface to a second volume to follow *Protein and Peptide Analysis by Mass* What has happened in between these times to make this second venture worthwhile New types of mass spectrometric instrumentation have appeared so that new techniques have become possible and existing techniques have become much more feasible More particularly however the newer ionization techniques introduced for the analysis of high molecular weight materials have now been thoroughly used and studied As a result there has been an enormous improvement in the associated sample handling technology so that these methods are now routinely applied to much smaller sample amounts as well as to more intractable samples Again this particular community of mass spectrometry users has both increased in number and diversified And riding this wave of acceptance leaders in the field have set their sights on more complex problems molecular interaction ion structures quantitation and kinetics are just a few of the newer areas reported in *Mass Spectrometry of Proteins and Peptides* As with the first volume one purpose of this collection *Mass Spectrometry of Proteins and Peptides* is to show the reader what can be done by the application of mass spectrometry and perhaps even to encourage the reader to venture down new paths **Matrix Metalloproteinase Protocols** Ian M. Clark, 2008-02-05 Research in the matrix metalloproteinase field began with the demonstration by Gross and Lapiere in 1962 that resorbing tadpole tail expressed an enzyme that could degrade collagen gels These humble beginnings have led us to the elucidation of around twenty distinct vertebrate MMPs along with a variety of homologs from such diverse organisms as sea urchin plants nematode worm and bacteria This coupled with four known specific inhibitors of MMPs the TIMPs gives a complex picture Part I of *Matrix Metalloproteinase Protocols* provides the reader with a selective overview of the MMP arena and a chance to come to grips with where the field has been where it is and where it is going I hope that this complements all of the methodology that comes later Part II presents the reader with a diverse set of methods for the expression and purification of MMPs and TIMPs bringing together the long and often hard earned experience of a number of researchers Part III allows the reader to detect MMPs and TIMPs at both the protein and mRNA level whereas Part IV gives the ability to assay MMP and TIMP activities in a wide variety of circumstances **Gene Knockout Protocols** Martin J. Tymms, Ismail Kola, 2008-02-03 As the major task of sequencing the human genome is near completion and full complement of human genes are catalogued attention will be focused on the ultimate goal to understand the normal biological functions of these genes and how alterations lead to disease states In this task there is a severe limitation in working with human material but the mouse has been adopted as the favored animal model because of the available genetic resources and the highly conserved gene conservation linkage organization In just of ten years since the first gene targeting experiments were performed in embryonic stem ES cells and mutations transmitted through the mouse germline more than a thousand mouse strains have been created These achievements have been made possible by pioneering work that showed that ES cells derived from preimplantation mouse embryos could be

cultured for prolonged periods without differentiation in culture and that homologous recombination between targeting constructs and endogenous DNA occurred at a frequency sufficient for recombinants to be isolated. In the next few years the mouse genome will be systematically altered and the techniques for achieving manipulations are constantly being streamlined and improved.

Transcription Factor Protocols Martin J. Tymms, 2008-02-03 The effort to sequence the human genome is now moving toward a conclusion. As all of the protein coding sequences are described an increasing emphasis will be placed on understanding gene function and regulation. One important aspect of this analysis is the study of how transcription factors regulate transcriptional initiation by RNA polymerase II which is responsible for transcribing nuclear genes encoding messenger RNAs. The initiation of Class II transcription is dependent upon transcription factors binding to DNA elements that include the core or basal promoter elements, proximal promoter elements and distal enhancer elements. General initiation factors are involved in positioning RNA polymerase II on the core promoter but the complex interaction of these proteins and transcriptional activators binding to DNA elements outside the core promoter regulate the rate of transcriptional initiation. This initiation process appears to be a crucial step in the modulation of mRNA levels in response to developmental and environmental signals. *Transcription Factor Protocols* provides step by step procedures for key techniques that have been developed to study DNA sequences and the protein factors that regulate the transcription of protein encoding genes. This volume is aimed at providing researchers in the field with the well detailed protocols that have been the hallmark of previous volumes of the *Methods in Molecular Biology* series.

Adrenergic Receptor Protocols Curtis A. Machida, 2008-02-03 Adrenergic receptors are important modulators in the sympathetic control of various metabolic processes in the central and peripheral nervous systems. These receptors are localized at multiple sites throughout the central nervous system (CNS) and serve as important regulators of CNS mediated behavior and neural functions including mood, memory, neuroendocrine control and stimulation of autonomic function. *Adrenergic Receptor Protocols* consists of 35 chapters dealing with various aspects of adrenergic receptor analyses including the use of genetic RNA protein expression, transactivator, second messenger, immunocytochemical, electrophysiological, transgenic and in situ hybridization approaches. This volume details the use of various methods to examine the adrenergic receptor system using aspects of the genetic flow of information as a guide. DNA, RNA, transactivator, protein expression, second messenger analyses, cellular analyses, transgenic, whole animal approaches. *Adrenergic Receptor Protocols* displays step by step methods for successful replication of experimental procedures and would be useful for both experienced investigators and newcomers in the field including those beginning graduate study or undergoing postdoctoral training. The Notes section contained in each chapter provides valuable troubleshooting guides to help develop working protocols for your laboratory. With *Adrenergic Receptor Protocols* it has been my intent to develop a comprehensive collection of modern molecular methods for analyzing adrenergic receptors. I would like to thank the many chapter authors for their contributions.

Unveiling the Power of Verbal Art: An Mental Sojourn through **Natural Killer Cell Protocols Cellular And Molecular Methods**

In a world inundated with displays and the cacophony of fast connection, the profound power and psychological resonance of verbal artistry often diminish in to obscurity, eclipsed by the regular onslaught of sound and distractions. Yet, set within the musical pages of **Natural Killer Cell Protocols Cellular And Molecular Methods**, a captivating perform of fictional beauty that impulses with natural thoughts, lies an remarkable journey waiting to be embarked upon. Written by a virtuoso wordsmith, this mesmerizing opus manuals readers on an emotional odyssey, delicately exposing the latent potential and profound influence embedded within the intricate web of language. Within the heart-wrenching expanse with this evocative evaluation, we shall embark upon an introspective exploration of the book is main subjects, dissect its interesting publishing fashion, and immerse ourselves in the indelible effect it leaves upon the depths of readers souls.

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Natural Killer Cell Protocols Cellular And Molecular Methods Introduction

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