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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

Robert A. Rush

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 BiologyTM 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 Chromatin Protocols Peter B. Becker,2008-02-03 More than 40 years after the discovery

of the nucleosome as the fun mental unit of chromatin the multifaceted problem of how variations in ch matin structure affect the activity of the eukaryotic genome has not been solved However during the past few years research on chromatin structure and fu tion has gained considerable momentum and impressive progress has been made at the level of concept development as well as filling in crucial detail The structure of the nucleosome has been visualized at unprecedented reso tion Powerful multisubunit enzymes have been identified that alter histone DNA interactions in ways that expose regulatory sequences to factors initi ing and regulating such nuclear processes as transcription Though the imp tance of posttranslational modifications of histones notably their acetylation has long been known the finding that a number of bona fide regulators increase transcription by acetylating nucleosomes has lent new support to the old idea that the process of gene regulation is intimately related to the nature of the chromatin environment A wealth of nonhistone proteins contribute to a continuum of structures with distinct biochemical properties and varying degrees of DNA condensation Perhaps the most important conclusion from a large number of studies is a fresh appreciation of the dynamic nature of chromatin structure the built in flexibility providing the basis for regulation *Eicosanoid Protocols* Elias A Lianos,2008-02-03 **Neuropeptide Y Protocols** Ambikaipakan Balasubramaniam,2008-02-05 The observation that neuropeptide Y NPY is the most abundant peptide present in the mammalian nervous system and the finding that it elicits the most powerful orexigenic signal have led to active investigations of the properties of the NPY family of hormones including peptide YY PYY and pancreatic polypeptide PP Nearly two decades of research have led to the identification of several NPY receptor subtypes and the development of useful receptor selective ligands Moreover these investigations have imp cated NPY in the pathophysiology of a number of diseases including feeding disorders seizures memory loss anxiety depression and heart failure Vigorous efforts are therefore continuing not only to understand the bioche cal aspects of NPY actions but also toward developing NPY based treatments for a variety of disorders To facilitate these efforts it was decided to produce the first handbook on NPY research techniques as part of the Methods in Molecular Biology Series In compiling Neuropeptide Y Protocols I have gathered contributions on techniques considered critical for the advancement of the NPY field from experts in various disciplines Each chapter starts with a brief introduction with Materials and Methods sections following The latter sections are presented in an easy to follow step by step format The last section of the chapter Notes highlights pitfalls and the maneuvers employed to overcome them This information not usually disseminated in standard research pub cations may prove extremely useful for investigators employing these te niques in NYP research Cytoskeleton Methods and Protocols Ray H. Gavin,2008-02-03 Over the past two decades experimental studies have solidified the int pretation 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 commu cation 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 o 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 po mer 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 c pound 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 sociated with these agents and methods for doing so are presently being veloped 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 syst atic 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 mune complexes and others so that texts now describe three activation pa ways 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 implemen tion 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 stimul ing 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 te niques 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 c bining 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 fu tion of connexin channels Connexin Methods and Protocols is divided into three main parts

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 diff ent 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 p tein 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 inc sion 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 p cesses involving DNA including DNA replication transcription and rec bination 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 und standing 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 st 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 recombi tion 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 dis plines 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 descr tions of the techniques and methods needed by researchers operating across the field sdiverse areas The present volume Bacterial Toxins Methods and Pro cols 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 s tion 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 t sues and for nuclease based therapeutics **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 h ever 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 org isms for developmental studies In Molecular Methods in Developmental Bi ogy 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 transp ent embryos of zebrafish render them well suited for in situ hybridization and immunohistochemistry and good for observing mutations in genetic screens **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 interc nected 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 impu ties 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 te niques introduced for the analysis of high molecular weight materials have now been thoroughly used and studied As a result there has been an en mous 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 Spectr etry 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 Lapi re 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

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