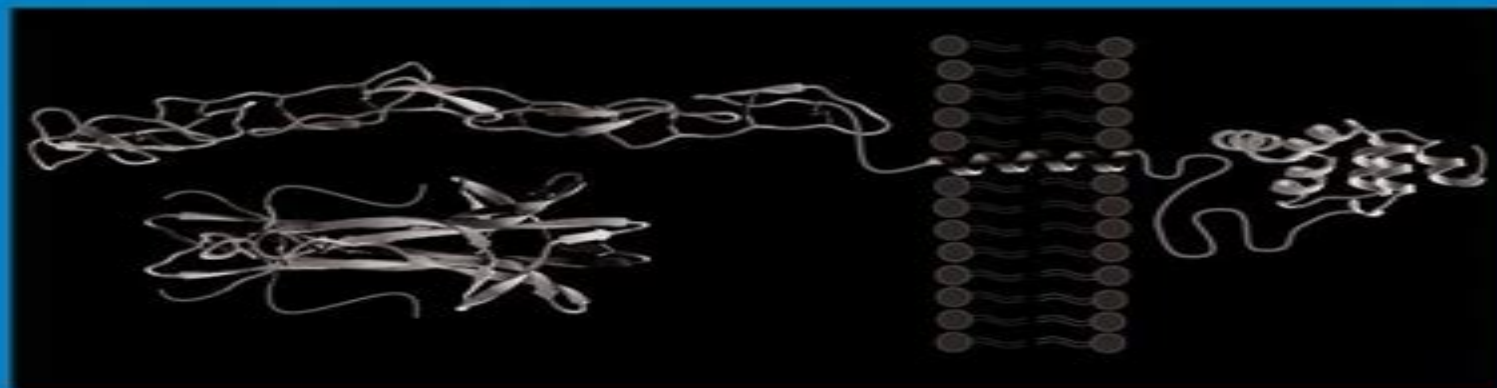


Methods in Molecular Biology™

VOLUME 169

Neurotrophin Protocols

Edited by
Robert A. Rush



HUMANA PRESS

Neurotrophin Protocols Methods In Molecular Biology S

Ian M. Clark



Neurotrophin Protocols Methods In Molecular Biology S:

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

The ELISA Guidebook John R. Crowther, 2008-02-04 John R Crowther provides today's premier practical guide to the understanding and application of ELISA Updating and greatly expanding his widely appreciated earlier publication *ELISA Theory and Practice* 1995 this important work introduces chapters on such major new topics as checkerboard titrations quality control of testing kit production and control novel monoclonal antibodies validation of assays statistical requirements for data examination and epidemiological considerations With its numerous worked examples detailed instructions and extensive illustrations *The ELISA Guidebook* offers a powerful synthesis of all the basic concepts and practical experimental details investigators need to understand develop and apply the new ELISA methodology successfully in day to day basic and clinical 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.

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 outgrowth 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.

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

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

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 well 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

Proteoglycan Protocols Renato V. Iozzo, 2008-02-02 Proteoglycans are some of the most elaborate macromolecules of

mammalian and lower organisms The covalent attachment of at least five types of glycosaminoglycan side chains to more than forty individual protein cores makes these molecules quite complex and endows them with a multitude of biological functions Proteoglycan Protocols offers a comprehensive and up to date collection of preparative and analytical methods for the in depth analysis of proteoglycans Featuring step by step detailed protocols this book will enable both novice and experienced researchers to isolate intact proteoglycans from tissues and cultured cells to establish the composition of their carbohydrate moieties to generate strategies for prokaryotic and eukaryotic expression to utilize methods for the suppression of specific proteoglycan gene expression and for the detection of mutant cells and degradation products and to study specific interactions between proteoglycans and extracellular matrix proteins as well as growth factors and their receptors The readers will find concise yet comprehensive techniques carefully drafted by leading experts in the field Each chapter commences with a general Introduction followed by a detailed Materials section and an easy to follow Methods section An asset of each chapter is the extensive notation that includes troubleshooting tips and practical considerations that are often lacking in formal methodology papers The reader will find this section most valuable because it is clearly provided by experienced scientists who have first hand knowledge of the techniques they outline In addition most of the chapters are well illustrated with examples of typical data generated with each method

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

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.

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.

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.

Genomics Protocols Michael P. Starkey, Ramnath Elaswarapu, 2008-02-03 We must unashamedly admit that a large part of the motivation for editing **Genomics Protocols** was selfish. The possibility of assembling in a single volume a unique and comprehensive collection of complete protocols relevant to our work and the work of our colleagues was too good an opportunity to miss. We are pleased to report however that the outcome is something of use not only to those who are experienced practitioners in the genomics field but is also valuable to

the larger community of researchers who have recognized the potential of genomics research and may themselves be beginning to explore the technologies involved Some of the techniques described in Genomics Protocols are clearly not restricted to the genomics field indeed a prerequisite for many procedures in this discipline is that they require an extremely high throughput beyond the scope of the average investigator However what we have endeavored here to achieve is both to compile a collection of procedures concerned with geno scale investigations and to incorporate the key components of bottom up and top down approaches to gene finding The technologies described extend from those traditionally recognized as coming under the genomics umbrella touch on proteomics the study of the expressed protein complement of the genome through to early therapeutic approaches utilizing the potential of genome programs via gene therapy Chapters 27 30

MHC Protocols Stephen H. Powis, Robert W. Vaughan, 2008-02-05 The aim of MHC Protocols is to document protocols that can be used for the analysis of genetic variation within the human major histocompatibility complex MHC HLA region The human MHC encompasses approximately 4 million base pairs on the short arm of chromosome 6 at cytogenetic location 6p21.3 The region is divided into three subregions The telomeric class I region contains the genes that encode the HLA class I molecules HLA A B and C The centromeric class II region contains the genes encoding the HLA class II molecules HLA DR DQ and DP In between is the class III region originally identified because it contains genes encoding components of the complement pathway The entire human MHC has recently been sequenced and each subregion is now known to contain many other genes a number of which have immunological functions The study of polymorphism within the MHC is well established because the region contains the highly polymorphic HLA genes HLA polymorphism has been used extensively in solid organ and bone marrow transplantation to match donors and recipients As a result large numbers of HLA alleles have been identified a process that has been further driven by recent interest in HLA gene diversity in ethnic populations The extreme genetic variation in HLA genes is believed to have been driven by the evolutionary response to infectious agents but relatively few studies have analyzed associations between HLA genetic variation and infectious disease which has been difficult to demonstrate

DNA-Protein Interactions Tom Moss, 2008-02-02 Dr Tom Moss assembles the new standard collection of cutting edge techniques to identify key protein DNA interactions and define their components their manner of interaction and their manner of function both in the cell and in the test tube The techniques span a wide range from factor identification to atomic detail and include multiple DNA footprinting analyses including in vivo strategies gel shift EMSA optimization SELEX surface plasmon resonance site specific DNA protein crosslinking and UV laser crosslinking Comprehensive and broad ranging DNA Protein Interactions Principles and Protocols 2nd Edition offers a stellar array of over 100 up to date and readily reproducible techniques that biochemists and molecular cellular and developmental biologists can use successfully today to understand DNA protein interactions

Adipose Tissue Protocols Gérard Ailhaud, 2008-02-03 Adipose tissue is recognized to be exquisitely sensitive to hormone action and is also now recognized as a

secretory and endocrine organ required for reproduction and good health Adipocytes are smart cells able within the tissue to communicate with surrounding cells but also with various organs particularly via leptin acting on the central nervous system Brown adipose tissue BAT and white adipose tissue WAT are known to be distinct tissues whereas the heterogeneity of WAT depots is well established Unfortunately excess WAT leads to obesity which is the most common health problem in industrialized countries Therefore from both a scientific and a technical point of view the time has come to create a survey of adipose tissues and their neglected adipocytes In Adipose Tissue Protocols I have attempted to gather together chapters from all areas of adipose tissue research from in vivo to in vitro studies and to provide methods covering a wide variety of techniques including the choice of adipose tissue depot and of morphological techniques for the study of BAT and WAT the isolation subcellular fractionation and transfection of adipocytes where the low density of these cells must be taken into account assays of nutrient and ion fluxes and the metabolic aspects of nutrient uptake assays of lipid related enzymes biopsies and quantification of lipid related mRNAs cultures of adipose precursor cells from WAT and BAT of various species including human tissue measurements of adipose secretory products and assessment of WAT metabolism in vivo

Superantigen Protocols Teresa Krakauer, 2008-02-05 Leading researchers in the biological chemical and physical investigation of superantigens describe in step by step detail their best experimental techniques to assess the physical characteristics and biological effects of superantigens Their protocols range from those for investigating the interactions of superantigens with cellular receptors to those for the analysis of their immunological and biological effects including methods for using BIOcore to determine binding kinetics and establishing various lymphocyte cell culture systems There are also accounts of such methods as the RNase protection assay cytokine ELISA FACS analysis and cytokine production at the single cell level

Two-Hybrid Systems Paul N. MacDonald, 2008-02-03 Paul N MacDonald has assembled a collection of powerful molecular tools for examining and characterizing protein protein protein DNA and protein RNA interactions The techniques range from the most basic introducing plasmids into yeasts interaction assays and recovering the plasmids from yeast to the most advanced alternative strategies involving one hybrid split two hybrid three hybrid membrane recruitment systems and mammalian systems Methods are also provided for dealing with the well known problem of artifacts and false positives and for identifying the interacting partners in important biological systems including the SMAD and nuclear receptor pathways To ensure ready reproducibility and robust results each technique is described in step by step detail by researchers who employ it regularly

Protein Structure, Stability, and Folding Kenneth P. Murphy, 2008-02-04 In Protein Structure Stability and Folding Kenneth P Murphy and a panel of internationally recognized investigators describe some of the newest experimental and theoretical methods for investigating these critical events and processes Among the techniques discussed are the many methods for calculating many of protein stability and dynamics from knowledge of the structure and for performing molecular dynamics simulations of protein unfolding New experimental approaches presented

include the use of co solvents novel applications of hydrogen exchange techniques temperature jump methods for looking at folding events and new strategies for mutagenesis experiments Unique in its powerful combination of theory and practice Protein Structure Stability and Folding offers protein and biophysical chemists the means to gain a more comprehensive understanding of some of this complex area by detailing many of the major techniques in use today Capillary Electrophoresis of Nucleic Acids Keith R. Mitchelson, Jing Cheng, 2008-02-05 The development of PCR which enables extremely small amounts of DNA to be amplified led to the rapid development of a multiplicity of analytical procedures that permit use of this new resource for the analysis of genetic variation and for the detection of disease causing mutations The advent of capillary electrophoresis CE with its power to separate and analyze very small amounts of DNA has also stimulated researchers to develop analytical procedures for the CE format The advantages of CE in terms of speed and reproducibility of analyses are manifold Furthermore the high sensitivity of detection and the ability to increase sample throughput with parallel analysis has led to the creation of a full range of analysis of DNA molecules from modified DNA adducts and single strand oligonucleotides through PCR amplified DNA fragments and whole chromosomes Capillary Electrophoresis of Nucleic Acids focuses on analytical protocols that can be used for detection and analysis of mutations and modification from precise DNA loci through entire genomes of organisms Important practical considerations for CE such as the choice of separation media electrophoresis conditions and the influence of buffer additives and dyes on DNA mobility are discussed in several key chapters and within particular applications

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