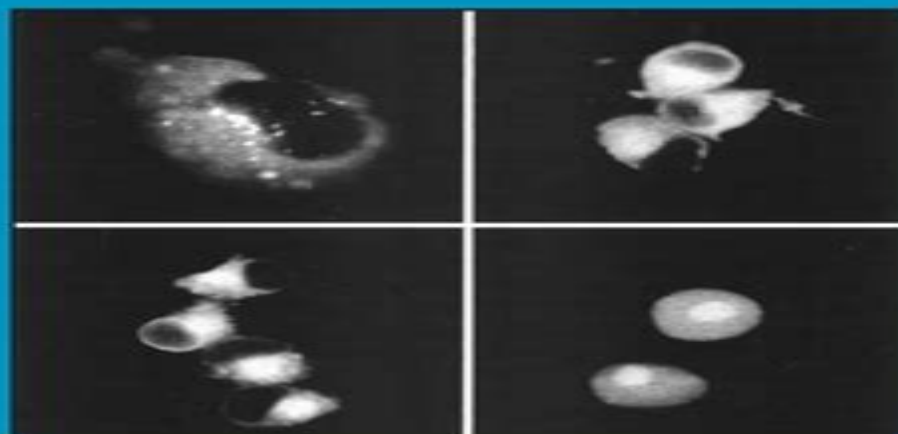


Methods in Molecular Biology™

VOLUME 124

Protein Kinase Protocols

Edited by
Alastair D. Reith



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Protein Kinase Protocols

Wafik S. El-Deiry



Protein Kinase Protocols:

Protein Kinase Protocols Alastair D. Reith, 2008-02-02 As key components of many cell signaling pathways protein kinases are implicated in a broad variety of diseases including cancers and neurodegenerative conditions and offer considerable potential as tractable targets for therapeutic intervention In Protein Kinase Protocols a panel of highly skilled laboratory investigators describe both basic and more sophisticated methods for the analysis of kinase mediated signaling cascades with emphasis on the identification of proteins according to their interactive relationships and the analysis of their functional properties Described in step by step detail these readily reproducible techniques offer novices quick access to a complicated field and provide more experienced investigators many novel time saving ploys Emphasis is given to the critical technical steps that are often omitted from methods published in the primary literature There are also tips on potential pitfalls and copious notes on how to adjust the protocols to work in related systems Broad in its range of techniques and thoroughly detailed to help ensure experimental success Protein Kinase Protocols offers both novice and experienced investigators powerful tools for understanding the functional roles of specific protein kinases within signaling cascades and for identification and evaluation of novel therapeutic targets

Protein Kinase C Protocols Alexandra C. Newton, 2008-02-03 Since the discovery that protein kinase C PKC transduces the abundance of signals that result in phospholipid hydrolysis this enzyme has been at the forefront of research in signal transduction Protein Kinase C Protocols covers fundamental methods for studying the structure function regulation subcellular localization and macromolecular interactions of PKC Protein Kinase C Protocols is divided into 11 sections representing the major aspects of PKC regulation and function Part I contains an introduction and a historical perspective on the discovery of PKC by Drs Yasutomi Nishizuka and Ushio Kikkawa Part II describes methods to purify PKC Part III describes the standard methods for measuring PKC activity its enzymatic activity and its stimulus dependent translocation from the cytosol to the membrane Part IV describes methods for measuring the membrane interaction of PKC in vivo and in vitro Part V provides methodologies and techniques for measuring the phosphorylation state of PKC including a protocol for measuring the activity of PKC's upstream kinase PDK 1 Novel methods for identifying substrates are described in Part VI Part VII presents protocols for expressing and analyzing the membrane targeting domains of PKC Part VIII provides a comprehensive compilation of methods used to identify binding partners for PKC Part IX describes pharmacological probes used to study PKC The book ends with a presentation of genetic approaches to study PKC Part X and a discussion of approaches used to study PKC in disease Part XI

MAP Kinase Signaling Protocols Rony Seger, 2008-02-03 Mitogen activated protein kinase MAPK signaling cascades are a group of protein kinases that play a central role in the intracellular transmission of extracellular signals These cascades operate as major lines of communication within a complicated signaling network that regulates many cellular processes including proliferation differentiation development stress response and apoptosis More than 15 000 papers on MAPKs have been published over the

past few years with the number of publications increasing each year More and more laboratories embark on the study of MAPK cascades in many distinct cellular systems and in particular their role in disease Future challenges in the study of MAPK cascades remain in understanding the role of the various components and isoforms of the cascades in the multiple critical functions that they regulate in the whole organism as well as the diseases caused by their malfunction Data from gene disrupted mice suggest that inhibition of the MAPK cascades may have serious consequences on the development and growth of the animals For example targeted deletion of MEK1 is lethal owing to developmental problems of placental vasculature and abnormal fibroblast migration This lethality occurs in spite of the normal expression of MEK2 indicating that although the two MEK isoforms are apparently similar they do have distinct functions at least during embryogenesis The ERK cascade was also shown to play a central role in brain function and in learning and memory

Signal Transduction Protocols Robert C. Dickson, Michael D. Mendenhall, 2008-02-02 In 1995 *Signal Transduction Protocols* edited by David A Kendall and Stephen J Hill was published in the *Methods in Molecular Biology* series This second edition represents an update to that previous work with an emphasis on new methodologies that have developed in the last few years The goal then and now is to provide procedures written by experts with first hand experience in a detail that goes far beyond what is generally encountered in the methods section of most journals and thus actually permits a particular procedure to be replicated In addition we have had as a secondary goal the identification of protocols for the assay of general classes of signal transduction components that ideally can be adapted to the assay of any member of that class The ability to do this has resulted in large part from the use of affinity based assays the ease with which specific proteins can be specifically tagged and an explosion in the availability of highly specific antibodies from commercial sources especially antibodies raised against signaling proteins of human origin The number of available approaches is fortunately for those working in signaling research far too great to fit within the confines of this volume so hard choices as to what to include had to be made

The Proteomics Protocols Handbook John M. Walker, 2007-10-09 Hands on researchers describe in step by step detail 73 proven laboratory methods and bioinformatics tools essential for analysis of the proteome These cutting edge techniques address such important tasks as sample preparation 2D PAGE gel staining mass spectrometry and post translational modification There are also readily reproducible methods for protein expression profiling identifying protein protein interactions and protein chip technology as well as a range of newly developed methodologies for determining the structure and function of a protein The bioinformatics tools include those for analyzing 2D GEL patterns protein modeling and protein identification All laboratory based protocols follow the successful *Methods in Molecular Biology*™ series format each offering step by step laboratory instructions an introduction outlining the principle behind the technique lists of the necessary equipment and reagents and tips on troubleshooting and avoiding known pitfalls

Methods in Molecular Biology: Protein kinase C protocols John M. Walker, 1984 ***Methods in Molecular Biology: Protein kinase protocols***

John M. Walker, 1984 **B Cell Protocols** Hua Gu, Klaus Rajewsky, 2008-02-04 B lymphocyte development and function remains an exciting area of research for those interested in the physiology and pathology of the immune system in higher animals. While recent advances in genetics and cellular and molecular biology have provided a large spectrum of powerful new experimental tools in this field, it is both time consuming and often very difficult for a student or just any bench side worker to identify a reliable experimental protocol in the ocean of the literature. The aim of B Cell Protocols is to provide a collection of diverse protocols ranging from the latest inventions and applications to some classic but still frequently used methods in B cell biology. The authors of the various chapters are all highly qualified scientists who are either the inventors or expert users of these methods. Their extensive experience in mastering a particular method provides not only the step by step details of a reproducible protocol but also useful troubleshooting tips that readers will appreciate in their daily work. We hope that this book will be helpful for both beginning and experienced researchers in the field in designing or modifying an experimental approach and exploring a biological question from multiple angles.

Transmembrane Signaling Protocols Dafna Bar-Sagi, 1998 This collection of practical cutting edge techniques for the study of cell signaling provides detailed step by step instructions, helpful notes and troubleshooting tips that make even the most powerful of the newest techniques readily reproducible. The protocols presented include the use of peptide libraries to study transmembrane signaling, the use of single cell assays to analyze signal transduction pathways, the reconstitution of signaling complexes, methods for analyzing protein protein interactions and more. Introductory reviews explain the basic theory and enable researchers new to the area to rapidly gain understanding as well as command of the practical knowledge and expertise afforded by the protocols. Transmembrane Signaling Protocols makes available to all researchers the many state of the art techniques that have recently led to landmark discoveries in transmembrane signaling.

Flavoprotein Protocols Steven K. Chapman, Graeme A. Reid, 2008-02-03 As a scientist with an interest in proteins, you will at some time in your career isolate an enzyme that turns out to be yellow or perhaps you already have. Alternatively, you may identify a polypeptide sequence that is related to known flavin containing proteins. This may or may not be your first encounter with flavoproteins. However, even if you are an old hand in the field, you may not have exploited the full range of experimental approaches applicable to the study of flavoproteins. We hope that Flavoprotein Protocols will encourage you to do so. In this volume, we have sought to bring together a range of experimental methods of value to researchers with an interest in flavoproteins, whether or not these researchers have experience in this area. A broad range of techniques from the everyday to the more specialized is described by scientists who are experts in their fields and who have extensive practical experience with flavoproteins. The wide range of approaches from wet chemistry to dry computation has as a consequence demanded a range of formats. Where appropriate, particularly for analytical methods, the protocol described is laid out in easy to follow steps. In other cases, e.g. the more advanced spectroscopies and computational methods, it is far more apt to describe the general approach and relevance of the

methods We hope this wide ranging approach will sow the seeds of many future collaborations between laboratories and further our knowledge and understanding of how proteins work

Phosphodiesterase Methods and Protocols Claire Lugnier, 2008-02-04 Research leaders in the PDE field describe new concepts and techniques for investigating the role of PDEs in orchestrating normal and pathophysiological responses Presented in step by step detail these readily reproducible methods allow the measurement of cyclic nucleotide variations in living cells as well as their visualization in a spatio temporal manner the localization and characterization of their activities in tissues and living cells and the assessment of targeted PDEs in creating specific tools and drugs

Chromatin Protocols Peter B. Becker, 2008-02-03 More than 40 years after the discovery of the nucleosome as the fundamental unit of chromatin the multifaceted problem of how variations in chromatin structure affect the activity of the eukaryotic genome has not been solved However during the past few years research on chromatin structure and function 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 resolution Powerful multisubunit enzymes have been identified that alter histone DNA interactions in ways that expose regulatory sequences to factors initiating and regulating such nuclear processes as transcription Though the importance 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

Short Protocols in Molecular Biology Frederick M. Ausubel, 2002 Recently expanded to 2 volumes Short Protocols in Molecular Biology Fifth Edition provides condensed descriptions of more than 700 methods compiled from Current Protocols in Molecular Biology Includes new chapters on chromatin assembly and analysis nucleic acid arrays generation and use of combinatorial libraries discovery and analysis of differentially expressed genes in single cells and cell populations The book is specifically designed to provide quick access to step by step instructions for the essential methods used in every major area of molecular biological research Short Protocols in Molecular Biology Fifth Edition is an authoritative and indispensable guide for all life scientists researchers and students at the graduate and advanced undergraduate level Expanded to 2 volumes

Novel Anticancer Drug Protocols John K. Buolamwini, Alex A. Adjei, 2008-02-01 We are in an exciting era in the war against cancer with real prospects for novel anticancer drugs that are cancer cell specific without the toxicities that have been the hallmark of conventional cytotoxic cancer chemotherapy Advances in cancer cell biology fueled by the molecular biology revolution have resulted in the uncovering of many novel potential molecular targets for cancer therapy New anticancer drug discovery and

development is now largely focused on exploiting these new molecular targets which encompass oncogenes tumor suppressor genes and their gene products as well as targets involved in tumor angiogenesis metastasis survival and longevity mechanisms Exploitation of some of these targets has already yielded fruits and introduced new paradigms of molecularly targeted cancer therapy into the clinic namely protein kinase inhibition by antibodies or small molecules exemplified by Herceptin trastuzumab a humanized antibody targeted against the HER 2 growth factor receptor tyrosine kinase for the treatment of metastatic breast cancer and Gleevec a small molecule bcr abl kinase inhibitor for the treatment of chronic myelogenous leukemia

Trinucleotide Repeat Protocols Yoshinori Kohwi, 2008-02-03 Trinucleotide repeats are relatively common in the human genome These simple repeats have received much attention since epoch making discoveries were made that particular trinucleotide repeats are expanded in the causal genes of human hereditary neurological disorders For example the CGG repeat is expanded in fragile X syndrome at the 5' untranslated region UTR of its causal gene In myotonic dystrophy it is the CTG repeat that is expanded at the 3' UTR of its causal gene The CAG repeat was also found expanded in coding regions of the genes responsible for X linked spinal and bulbar muscular atrophy Huntington's disease spinocerebellar ataxia and other disorders On the other hand expansion of the GAA repeat was identified in the intron of the gene responsible for the Friedreich's ataxia For these trinucleotide repeat diseases the longer the trinucleotide expansion the earlier the age of onset and the more severe the syndrome Thus these findings that showed the intriguing link between a particular trinucleotide expansion and its associated neurological disorders have led to a new field of intensive study Active research addressing the underlying mechanisms for trinucleotide repeat diseases has employed various approaches ranging from DNA biochemistry to animal models for the diseases In particular animal models for the triplet repeat diseases have provided excellent resources not only for understanding the mechanisms but also for exploring therapeutic interventions

Combinatorial Peptide Library Protocols Shmuel Cabilly, 2008-02-02 During the course of evolution an imbalance was created between the rate of vertebrate genetic adaptation and that of the lower forms of living organisms such as bacteria and viruses This imbalance has given the latter the advantage of generating relatively quickly molecules with unexpected structures and features that carry a threat to vertebrates To compensate for their weakness vertebrates have accelerated their own evolutionary processes not at the level of whole organism but in specialized cells containing the genes that code for antibody molecules or for T cell receptors That is when an immediate requirement for molecules capable of specific interactions arose nature has preferred to speed up the mode of Darwinian evolution in preference to any other approach such as the use of X ray diffraction studies and computergraphic analysis Recently Darwinian rules have been adapted for test tube research and the concept of selecting molecules having particular characteristics from random pools has been realized in the form of various chemical and biological combinatorial libraries While working with these libraries we noticed the interesting fact that when combinatorial libraries of oligopeptides were allowed to interact with different selector proteins

only the actual binding sites of these proteins showed binding properties whereas the rest of the protein surface seemed inert. This seemingly common feature of protein having no extra potential binding sites was probably selected during evolution in order to minimize nonspecific interactions with the surrounding milieu.

Methods in Plant Cell Biology David W. Galbraith, Hans J. Bohnert, Don P. Bourque, 1995. *Methods in Plant Cell Biology* provides in two volumes a comprehensive collection of analytical methods essential for researchers and students in the plant sciences. Individual chapters written by experts in the field provide an introductory overview followed by a step by step technical description of the methods. Key Features: Written by experts many of whom have developed the individual methods described. Contains most if not all the methods needed for modern research in plant cell biology. Up to date and comprehensive. Full references. Allows quick access to relevant journal articles and to the sources of chemicals required for the procedures. Selective concentration on higher plant methods allows for particular emphasis on those problems specific to plants.

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.

Ribozymes and siRNA protocols Mouldy Sioud, 2008-02-03. In this completely updated and expanded edition of a classic bench manual hands on experts take advantage of the latest advances in ribozyme, DNAzyme, hammerhead ribozymes and derivatives and RNA interference technologies to describe in detail the exciting and successful methods now available for gene inactivation in vitro and in vivo. Their optimized techniques employ hairpin ribozymes, DNAzymes, hammerhead ribozymes and derivatives, group I intron ribozymes, RNase P ribozymes and siRNAs as well as general methods for RNA structure analysis, delivery of oligonucleotides and gene therapy. Also provided are novel methods for identifying accessible cellular mRNA sites, group I intron and RNase P ribozyme protocols for effective design, selection and therapeutic applications and the latest RNAi methods for sequence specific gene silencing in a wide variety of organisms. Additional techniques cover the analysis of ribozyme structures and

conformational transitions using nucleotide analog interference mapping and fluorescence resonance energy transfer the use of ribozymes in clinical and gene therapy and the use of ribozymes and DNazymes in rodent models of human disease Each proven protocol includes a background introduction outlining the principle behind the technique step by step instructions lists of equipment and reagents and tips on troubleshooting and avoiding known pitfalls Comprehensive and up to date Ribozymes and siRNA Protocols details for experienced and novice investigators alike the many exciting advances in our understanding of nucleic acid enzymes as well as demonstrating how they may be used to analyze gene function and target validation and to productively develop novel therapeutics for human diseases

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 has ever 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

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