

Methods in  
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Springer Protocols



Alexander E. Kalyuzhny *Editor*

# Handbook of ELISPOT

Methods and Protocols

*Fourth Edition*

 Humana Press

# Handbook Of Elispot Methods And Protocols Methods In Molecular Biology

**Ian A. Darby, Tim D. Hewitson**



## **Handbook Of Elispot Methods And Protocols Methods In Molecular Biology:**

**Handbook of ELISPOT** Alexander E. Kalyuzhny, 2008-02-02 In this first book dedicated entirely to the ELISPOT a critical enzyme linked immunospot assay used widely in biomedical research recognized experts with first hand experience detail how to design perform and analyze these assays The readily reproducible techniques they provide cover a wide variety of topics including the use of membrane backed plates the standardization and validation procedures the removal of cells from ELISPOT plates cell separation techniques and the quantification of ELISPOT data There are also numerous ELISPOT applications involving animal models human cells measles multiple sclerosis immune responses multicytokine detection systems and immunocytochemistry Highlights include dual color and multiplex ELISPOT assays use of the ELISPOT assay on feline lymphocytes standardization of the ELISPOT procedure and combining the ELISPOT assay with immunohistochemistry

*DNA Viruses* Paul M. Lieberman, 2008-02-04 A compendium of readily reproducible and novel methods to manipulate DNA viruses and characterize their varied biological properties The authors emphasize techniques for viral detection and genetics but also include methods for structure determination gene expression replication pathogenesis complex cellular models recombinant genetics and computational systems approaches Wide ranging and highly practical DNA Viruses Methods and Protocols will stimulate new directions in virology research with its novel strategies for engineering viral vectors in gene therapy and its advanced approaches for detecting viruses in human disease

**Cell Cycle Control** Tim Humphrey, Gavin Brooks, 2008-02-04 The fundamental question of how cells grow and divide has perplexed biologists since the development of the cell theory in the mid 19th century when it was recognized by Virchow and others that all cells come from cells In recent years considerable effort has been applied to the identification of the basic molecules and mechanisms that regulate the cell cycle in a number of different organisms Such studies have led to the elucidation of the central paradigms that underpin eukaryotic cell cycle control for which Lee Hartwell Tim Hunt and Paul Nurse were jointly awarded the Nobel Prize for Medicine and Physiology in 2001 in recognition of their seminal contributions to this field The importance of understanding the fundamental mechanisms that modulate cell division has been reiterated by relatively recent discoveries of links between cell cycle control and DNA repair growth cellular metabolism development and cell death This new phase of integrated cell cycle research provides further challenges and opportunities to the biological and medical worlds in applying these basic concepts to understanding the etiology of cancer and other proliferative diseases

*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

*Biochemistry and*

*Molecular Biology Compendium* Roger L. Lundblad, 2019-11-11 This book is an accessible resource offering practical information not found in more database oriented resources The first chapter lists acronyms with definitions and a glossary of terms and subjects used in biochemistry molecular biology biotechnology proteomics genomics and systems biology There follows chapters on chemicals employed in biochemistry and molecular biology complete with properties and structure drawings Researchers will find this book to be a valuable tool that will save them time as well as provide essential links to the roots of their science Key selling features Contains an extensive list of commonly used acronyms with definitions Offers a highly readable glossary for systems and techniques Provides comprehensive information for the validation of biotechnology assays and manufacturing processes Includes a list of Log P values water solubility and molecular weight for selected chemicals Gives a detailed listing of protease inhibitors and cocktails as well as a list of buffers *Arabidopsis Protocols, 2nd Edition* Julio Salinas, Jose J. Sanchez-Serrano, 2008-02-04 For several decades *Arabidopsis thaliana* has been the organism of choice in the laboratories of many plant geneticists physiologists developmental biologists and biochemists around the world During this time a huge amount of knowledge has been acquired on the biology of this plant species which has resulted in the development of molecular tools that account for much more efficient research The significance that *Arabidopsis* would attain in biological research may have been difficult to foresee in the 1980s when its use in the laboratory started In the meantime it has become the model plant organism much the same way as *Drosophila* *Caenorhabditis* or mouse have for animal systems Today it is difficult to envision research at the cutting edge of plant biology without the use of *Arabidopsis* Since the first edition of *Arabidopsis Protocols* appeared new developments have fostered an impressive advance in plant biology that prompted us to prepare *Arabidopsis Protocols Second Edition* Completion of the *Arabidopsis* genome sequence offered for the first time the opportunity to have in hand all of the genetic information required for studying plant function In addition the development of whole systems approaches that allow global analysis of gene expression and protein and metabolite dynamics has encouraged scientists to explore new scenarios that are extending the limits of our knowledge

*Drug Allergy* Brian A. Baldo, Nghia H. Pham, 2020-12-08 The second edition of this book spans the broad range of modern therapeutic drugs from small molecules to biologic recombinant proteins It offers a comprehensive review of the classification and description of different drug induced systemic and cutaneous hypersensitivities an up to date coverage of individual culprit drugs in each group of therapeutics the diagnosis and mechanisms of reactions and important structure activity relationships New content expands to two areas of drug allergy that have recently experienced explosive growth biological therapies and new targeted chemotherapies Other new and expanded chapters address antimicrobials drugs used in anesthesia and surgery opioids non targeted anti cancer drugs vaccines and newly understood reaction mechanisms This new edition includes photographs of a wide variety of cutaneous manifestations that will be of use to other clinicians as well as allergists and dermatologists In addition to its wide clinical emphasis the book s mechanistic and structure activity detail

will provide valuable background for researchers and investigators in universities medical research institutes drug companies and regulatory agencies The second edition of Drug Allergy is an essential reference for practitioners across the medical disciplines from specialist clinicians surgeons GPs residents and medical students to nurses pharmacists dentists and those taking undergraduate and graduate courses in the biomedical sciences

**Embryonic Stem Cell Protocols** Kursad Turksen, 2008-02-04 Now in two volumes this completely updated and expanded edition of Embryonic Stem Cells Methods and Protocols provides a diverse collection of readily reproducible cellular and molecular protocols for the manipulation of nonhuman embryonic stem cells Volume one Embryonic Stem Cell Protocols Isolation and Characterization Second Edition provides a diverse collection of readily reproducible cellular and molecular protocols for the isolation maintenance and characterization of embryonic stem cells The second volume Embryonic Stem Cell Protocols Differentiation Models Second Edition covers state of the art methods for deriving many types of differentiating cells from ES cells Together the two volumes illuminate for both novices and experts our current understanding of the biology of embryonic stem cells and their utility in normal tissue homeostasis and regenerative medicine applications

**Peptide Synthesis and Applications** John Howl, 2008-02-02 Hands on experts describe in step by step detail the key methodologies of contemporary peptide synthesis and illustrate their numerous applications The techniques presented include protocols for chemical ligation the synthesis of cyclic and phosphotyrosine containing peptides lipoamino acid and sugar conjugated peptides and peptide purification and analyses Additional chapters detail methodologies and instrumentation for high throughput peptide synthesis many different applications of peptides as novel research tools and biological probes and the design and application of fluorescent substrate based peptides that can be used to determine the selectivity and activity of peptidases A practical guide to the identification of proteins using mass spectrometric analyses of peptide mixtures is also included

**Transmembrane Signaling Protocols** Hydar Ali, Haribabu Bodduluri, 2008-02-05 The previous edition of Transmembrane Signaling Protocols was published in 1998 Since then the human genome has been completely sequenced and new methods have been developed for the use of microarrays and proteomics to analyze global changes in gene expression and protein profiles These advances have increased our ability to understand transmembrane signaling processes in much greater detail They have also simultaneously enhanced our ability to determine the role of a large number of newly identified molecules in signaling events In addition novel video microscopy methods have been developed to image transmembrane signaling events in live cells in real time In view of these major advances it is time to update the previous edition Because of the success of that volume we have chosen to keep the essential character of the book intact Introductory chapters from experts have been included to provide overall perspective and an overview of recent advances in signal transduction pathways The individual chapters now include comprehensive detailed methods studies in genetically tractable systems fluorescence microscopy in live single cells ex vivo analysis of primary cells from transgenic mice as well as genomic and proteomic approaches to the analysis of

transmembrane signaling events We would like to express our deep gratitude to the coauthors of this publication We hope that Transmembrane Signaling Protocols Second Edition will serve as a valuable resource for future progress in the study of signal transduction pathways

**Cell Imaging Techniques** Douglas J. Taatjes, Brooke T. Mossman, 2008-02-04 A diverse collection of state of the art methods for the microscopic imaging of cells and molecules The authors cover a wide spectrum of complimentary techniques including such methods as fluorescence microscopy electron microscopy atomic force microscopy and laser scanning cytometry Additional readily reproducible protocols on confocal scanning laser microscopy quantitative computer assisted image analysis laser capture microdissection microarray image scanning near field scanning optical microscopy and reflection contrast microscopy round out this eclectic collection of cutting edge imaging techniques now available The authors also discuss preparative methods for particles and cells by transmission electron microscopy

**Differential Display Methods and Protocols** Peng Liang, Jonathan Meade, Arthur B. Pardee, 2008-02-04 Since the first edition of this book dedicated to differential display DD technology was published in 1997 we have witnessed an explosive interest in studying differential gene expression The gene hunting euphoria was initially powered by the invention of DD which was gradually overtaken by DNA microarray technology in recent years Then why is there still the need for second edition of this DD book First of all DD still enjoys a substantial lead over DNA microarrays in the ISI citation data see Table 1 despite the hundreds of millions of dollars spent each year on arrays This may come as a surprise to many but to us it implies that many of the DNA microarray studies went unpublished owing to their unfulfilled promises 1 Second unlike DNA microarrays DD is an open ended gene discovery method that does not depend on prior genome sequence information of the organism being studied As such DD is applicable to the study of all living organisms from bacteria fungi insects fish plants to mammals even when their genomes are not sequenced Second DD is more accessible technically and financially to most cost conscious cottage industry academic laboratories So clearly DD still has its unique place in the modern molecular biological toolbox for gene expression analysis

**Epidermal Growth Factor** Tarun B. Patel, Paul J. Bertics, 2008-02-05 A comprehensive collection of optimized methods for dissecting the mechanisms that control epidermal growth factors EGF and their regulators in both normal and pathological states These readily reproducible techniques range from the study of purified EGF receptor to complex signaling and processing networks in intact cells including a chapter on the clinical and pharmacological considerations of their use in cancer therapy The protocols follow the successful Methods in Molecular Biology™ series format each offering step by step laboratory instructions an introduction outlining the principles behind the technique lists of the necessary equipment and reagents and tips on troubleshooting and avoiding known pitfalls

**Cytochrome P450 Protocols** Ian R. Phillips, 2008-02-04 For this second edition of their much praised Cytochrome P450 the editors have collected accounts of the essential core techniques that use the latest methodologies for the investigation of P450s Highlights include protocols for spectral analysis and purification of P450s enzymatic assays of P450s and flavin

containing monooxygenases FMOs expression of P450s and FMOs in heterologous systems and the production and use of anti-peptide antibodies Additional chapters contain readily reproducible techniques for the transfection of hepatocytes for gene regulation studies P450 reporter gene assays in situ hybridization and analysis of genetic polymorphisms Although the emphasis is on P450s of mammalian origin many of the readily reproducible methods described are suitable for P450s from any source

**Nuclear Reprogramming** Steve Pells, 2008-02-04 A wide ranging collection of readily reproducible methods for performing nuclear reprogramming by nuclear transfer in several different species by fusion through both chemical treatment and electrically shocking cells and by in vivo treatment of cells with cell extracts Several methods of monitoring nuclear reprogramming are also presented including the use of transgenic markers activation of telomerase as an ES specific marker light and electron microscopic observation of structural changes in the nucleus and verification of surface marker expression and the differentiation potential of stem cells Biochemical methods are provided for the examination of chromatin protein modifications nucleosomal footprinting transcription factor binding and the study of DNA methylation changes both at the specific locus level and at the level of the whole nucleus

*Fluorescent Energy Transfer Nucleic Acid Probes* Vladimir V. Didenko, 2008-02-04 Fluorescent nucleic acid probes which use energy transfer include such constructs as molecular beacons molecular break lights Scorpion primers TaqMan probes and others These probes signal detection of their targets by changing either the intensity or the color of their fluorescence Not surprisingly these luminous multicolored probes carry more flashy names than their counterparts in the other fields of molecular biology In recent years fluorescent probes and assays which make use of energy transfer have multiplied at a high rate and have found numerous applications However in spite of this explosive growth in the field there are no manuals summarizing different protocols and fluorescent probe designs In view of this the main objective of *Fluorescent Energy Transfer Nucleic Acid Probes Designs and Protocols* is to provide such a collection Oligonucleotides with one or several chromophore tags can form fluorescent probes capable of energy transfer Energy transport within the probe can occur via the resonance energy transfer mechanism also called Förster transfer or by non-Förster transfer mechanisms Although the probes using Förster transfer were developed and used first the later non-Förster based probes such as molecular beacons now represent an attractive and widely used option The term fluorescent energy transfer probes in the title of this book covers both Förster based fluorescence resonance energy transfer FRET probes and probes using non-FRET mechanisms Energy transfer probes serve as molecule size sensors changing their fluorescence upon detection of various DNA reactions

Hormone Assays in Biological Fluids Michael J. Wheeler, William D. Fraser, J. S. Morley Hutchinson, 2008-02-04 Expert researchers who have developed and applied significant new assays describe in step by step detail a variety of methods for measuring a broad variety of hormones related peptides and synthetic steroids in various biological fluids The hormones measured range from glucocorticoids in biological fluids urinary steroids aldosterone in blood and plasma renin activity to gut hormones in plasma melatonin prolactin 6-sulfatoxymelatonin and

androgens in blood saliva and hair The emphasis is on noncommercial assays so that investigators can set up novel methods suited to their special needs Commercial assays are also described for comparative purposes Tutorials on radioimmunoassay gas chromatography mass spectrometry high performance liquid chromatography and PCR techniques help the reader to choose the best method for his or her purpose Handbook of Biochemistry and Molecular Biology ,2010-05-21 Edited by renowned protein scientist and bestselling author Roger L Lundblad with the assistance of Fiona M Macdonald of CRC Press this fourth edition of the Handbook of Biochemistry and Molecular Biology represents a dramatic revision the first in two decades of one of biochemistry s most referenced works This edition gathers a wealth of information not easily obtained including information not found on the web Offering a molecular perspective not available 20 years ago it provides physical and chemical data on proteins nucleic acids lipids and carbohydrates Presented in an organized concise and simple to use format this popular reference allows quick access to the most frequently used data Covering a wide range of topics from classical biochemistry to proteomics and genomics it also details the properties of commonly used biochemicals laboratory solvents and reagents Just a small sampling of the wealth of information found inside the handbook Buffers and buffer solutions Heat capacities and combustion levels Reagents for the chemical modification of proteins Comprehensive classification system for lipids Biological characteristics of vitamins A huge variety of UV data Recommendations for nomenclature and tables in biochemical thermodynamics Guidelines for NMR measurements for determination of high and low pKa values Viscosity and density tables Chemical and physical properties of various commercial plastics Generic source based nomenclature for polymers Therapeutic enzymes About the Editors Roger L Lundblad Ph D Roger L Lundblad is a native of San Francisco California He received his undergraduate education at Pacific Lutheran University and his PhD degree in biochemistry at the University of Washington After postdoctoral work in the laboratories of Stanford Moore and William Stein at the Rockefeller University he joined the faculty of the University of North Carolina at Chapel Hill He joined the Hyland Division of Baxter Healthcare in 1990 Currently Dr Lundblad is an independent consultant and writer in biotechnology in Chapel Hill North Carolina He is an adjunct Professor of Pathology at the University of North Carolina at Chapel Hill and Editor in Chief of the Internet Journal of Genomics and Proteomics Fiona M Macdonald Ph D F R S C Fiona M Macdonald received her BSc in chemistry from Durham University UK She obtained her PhD in inorganic biochemistry at Birkbeck College University of London studying under Peter Sadler Having spent most of her career in scientific publishing she is now at Taylor and Francis and is involved in developing chemical information products **Molecular Toxicology Protocols** Phouthone Keohavong,Stephen G. Grant,2008-02-03 A collection of cutting edge techniques for analyzing genotoxic exposure and detecting the resulting biological effects including endogenous metabolites up to and including the development of cancer The authors emphasize analytical methods that can be specifically applied to human populations and patients Among the applications detailed are the analysis of interactions between such cellular macromolecules as DNA and



proteins and chemical and physical agents the assessment of medically relevant toxicity and the characterization of genetic alterations induced in transgenic animals by in vivo systems There are also methods for the analysis of genotoxic exposure during gene expression of cytotoxicity caused by the induction of apoptosis of genetic alterations in reporter genes and oncogenes early premalignant detection of altered oncogenes and of individual variation in biotransformation and DNA repair capacity

*In Situ Hybridization Protocols* Ian A. Darby, Tim D. Hewitson, 2008-02-04 The technique of in situ hybridization in its various forms has been used routinely in many laboratories for a number of years In the post genome era gene arrays and proteomics have allowed us to identify hitherto unknown unrecognized pathways and mechanisms However rather than diminish the importance of in situ hybridization the now widespread use of screening technologies has increased the need to temporally and spatially localize the distribution of mRNA expression Our intention in *In Situ Hybridization Protocols* is to provide ample information for novices planning to set up the in situ hybridization technique and use it in their laboratory for the first time as well as giving updates of recent developments for those laboratories where in situ hybridization techniques are already in use Despite its widespread significance in situ hybridization has retained a reputation as one of the more difficult and capricious molecular biological techniques This may in part be because of the hybrid nature of the technique which often requires a mixture of molecular biological and histological skills The two techniques are usually taught and acquired in different streams of biological science The step by step and detailed protocols provided in *In Situ Hybridization Protocols* by researchers active in the field should make it possible for both the molecular biologist with little experience of histology and the histologist with little experience of molecular biology to use the techniques successfully in their laboratories

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