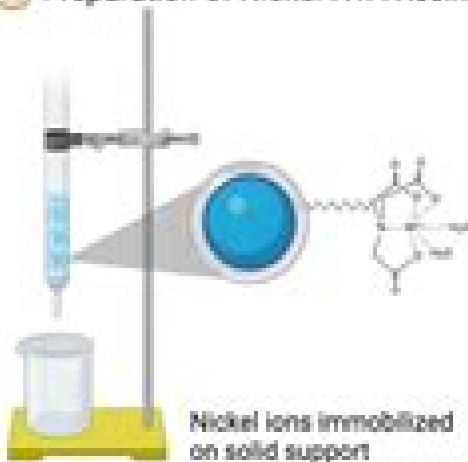


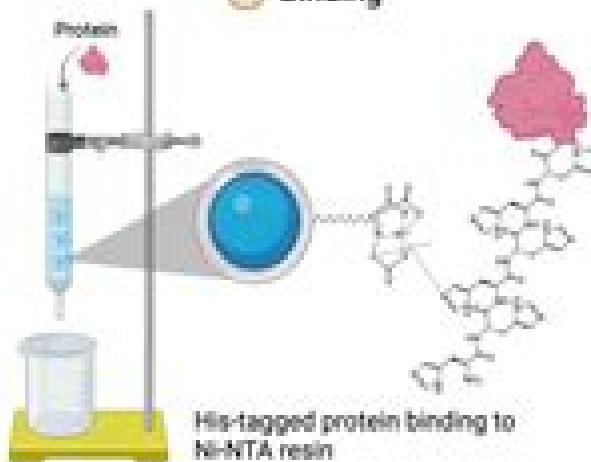
Ni-NTA His-Tag Protein Purification

1 Preparation of Nickel-NTA Resin



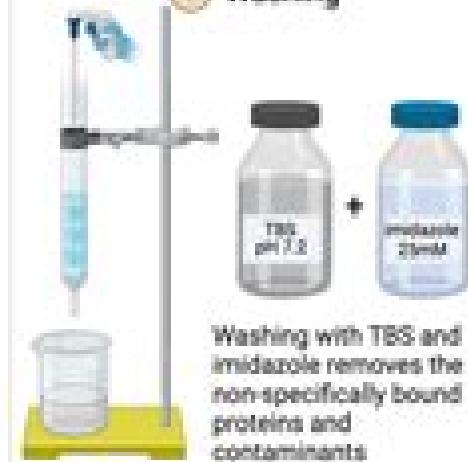
Nickel ions immobilized on solid support

2 Binding



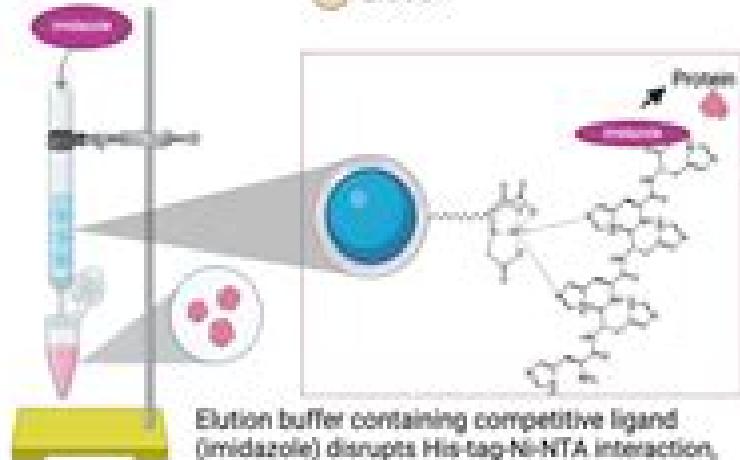
His-tagged protein binding to Ni-NTA resin

3 Washing



Washing with TBS and imidazole removes the non-specifically bound proteins and contaminants

4 Elution



Elution buffer containing competitive ligand (imidazole) disrupts His-tag-Ni-NTA interaction, releasing purified protein

5 Analysis



SDS-PAGE assesses the purity and integrity of the purified protein sample

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Regulators and Effectors of Small GTPases, Part F: Ras Family I ,2001-04-17 The critically acclaimed laboratory standard for more than 40 years Methods in Enzymology is one of the most highly respected publications in the field of biochemistry Since 1955 each volume has been eagerly awaited frequently consulted and praised by researchers and reviewers alike Now with more than 300 volumes all of them still in print the series contains much material still relevant today truly an essential publication for researchers in all fields of life sciences This volume and its companions Volumes 255 256 257 and the forthcoming 325 and 329 cover all biochemical and biological assays currently in use for analyzing the role of small GTPases in these aspects of cell biology at the molecular level

Cumulative Subject Index John N. Abelson,2002-11-27 The critically acclaimed laboratory standard for more than forty years Methods in Enzymology is one of the most highly respected publications in the field of biochemistry Since 1955 each volume has been eagerly awaited frequently consulted and praised by researchers and reviewers alike Now with more than 300 volumes all of them still in print the series contains much material still relevant today truly an essential publication for researchers in all fields of life sciences Supplements index volumes 33 75 95 120 140 175 199 229 265 285 and 320 Subject index Contributor index

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Screening Using Surface Plasmon Resonance Sameer Mahmood Zaheer,Ramachandraiah Gosu,2021-09-15 This volume describes methods and protocols for the fragment based screening of proteins using Surface Plasmon Resonance SPR The initial chapter of the book discusses the principle of SPR for the identification of biomolecular interactions while the subsequent chapters introduce methods for labelling proteins with different tags including histidine and biotin tags It also discusses techniques and factors that affect the amine and biotin streptavidin coupling and methods to optimize the interactions Next it describes fragment preparation for screening in SPR and presents methods to calculate equilibrium dissociation constant KD and ligand efficiency LE It reviews techniques of next generation injections that improve the efficiency of the characterization process over traditional SPR by determining the kinetics and affinity in a single step Finally the book elucidates a comprehensive yet representative description of challenges associated with the molecular interaction of proteins using SPR

Regulators and Effectors of Small GTPases, Part E: GTPases Involved in Vesicular Traffic ,2001-02-08 Small GTPases play a key role in many aspects of contemporary cell biology control of cell growth and differentiation regulation of cell adhesion and cell movement the organization of the actin cytoskeleton and the regulation of intracellular vesicular transport This volume and its companions Volumes 255 256 257 and the forthcoming 325 cover all biochemical and biological assays currently in use for analyzing the role of small GTPases in these aspects of cell biology at the molecular level

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and optimization The coverage includes various cell tissue and animal models Chapter 1 provides the foundations of cell biology of tight junction Chapter 2 covers the Biochemical approaches for paracellular channels and is followed by chapter 3 providing the Biophysical approaches Chapter 4 describes and discusses Histological approaches for tissue fixation and preparation Chapter 5 discusses Light microscopy while chapter 6 presents Electron microscopic approaches Chapter 7 covers Transgenic manipulation in cell cultures including DNA and siRNA Mutagenesis and viral infection Chapter 8 covers transgenic manipulation in mice including Knockout Knockin siRNA knockdown GFP LacZ reporter and overexpression The final chapter discusses the future developments of new approaches for tight junction research Researchers and advanced students in bioscience working on topics of cell junction ion channel and membrane protein will benefit from the described methods Clinicians and pathologists interested in tissue barrier diseases will also benefit from the biochemical and biophysical characterization of tight junctions in organ systems and their connection to human diseases Provides consistent and detailed research methods Covers various cell tissue and animal models Includes step by step guidance from beginner to sophisticated levels

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Weiner, Quinn Lu, 2002 Membrane Protein Structure Determination Jean-Jacques Lacapère, 2010-08-06 Membrane proteins representing nearly 40% of all proteins are key components of cells involved in many cellular processes yet only a small number of their structures have been determined Membrane Protein Structure Determination Methods and Protocols presents many detailed techniques for membrane protein structure determination used today by bringing together contributions from top experts in the field Divided into five convenient sections the book covers various strategies to purify membrane proteins approaches to get three dimensional crystals and solve the structure by x ray diffraction possibilities to gain structural information for a membrane protein using electron microscopy observations recent advances in nuclear magnetic resonance NMR and molecular modelling strategies that can be used either to get membrane protein structures or to move from atomic structure to a dynamic understanding of a molecular functioning mechanism Written in the highly successful Methods in Molecular Biology™ series format chapters include introductions to their respective topics lists of the necessary materials and reagents step by step readily reproducible laboratory protocols and tips on troubleshooting and avoiding known pitfalls Comprehensive and easy to use Membrane Protein Structure Determination Methods and Protocols serves as an ideal reference for scientists seeking to further our knowledge of these vital and versatile proteins as well as our overall understanding of the complicated world of cell biology

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