

METHODS IN MOLECULAR BIOLOGY™

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Free Radicals and Antioxidant Protocols

Second Edition

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Preface

Reactive oxygen and nitrogen species (RONs) affect normal physiological processes and pathological conditions. In spite of the development of analytical and technological advancements in the detection and determination of RONs, there is a growing demand to achieve more rapid and accurate detection and determination of RONs and also of redox stress. In order to satisfy such a demand, we chose to prepare this current volume which comprises 27 chapters contributed by world-renowned experts on the state-of-the-art analytical and technological aspects of the detection and determination of RONs, and oxidative, nitrative, nitrosative, and redox stresses in biological systems, *in vitro* and *in vivo*.

There is a need to establish biomarkers for the oxidative stress-induced genetic damage in cellular and animal models. This need is addressed herein by offering protocols for (1) the generation of stable oxidative stress-resistant phenotypes of Chinese hamsters fibroblasts, (2) *in vivo* detection and measurement of free radicals and oxygen, (3) *in vivo* determination of tissue and DNA damage as a result of radical exposure, and (4) *in vivo* and *in vitro* monitoring of footprints of free-radical and antioxidant reactions. In the sections dedicated to the analysis of antioxidants and their metabolites, we describe methods for the analysis of phenolic acids and flavonoids, eugenol antioxidants, and the recycling of ascorbic and lipoic acids. We also report on cellular reductive capacity, determination of glutathione, mitochondrial transmembrane potential, and cytotoxicity in cardiomyocytes under RONs stresses.

Lipids are at the epicenter of oxidative stress. However, the analysis of lipid peroxidation, either *in vitro* or *in vivo*, is still complex, and novel methods and technologies are always in great demand to accurately analyze the peroxidized lipids. This volume presents the recent advances in the soft (electro spray) ionization mass spectrometry (MS) of phospholipid hydroperoxides for cellular and tissue oxidative lipidomics, the simultaneous analysis of multiple lipid oxidation products *in vivo* by LC-MS, and protocols for enzyme immunoassay of isoprostanes and chemiluminescence determination of nitrite in plasma. We also discuss the preparation, purification, and characterization of lipoxygenase-catalyzed phosphatidylinositol peroxides, and the biology and chemistry of oxidized low-density lipoprotein.

Gene transfer has become a useful therapeutic strategy in the treatment of certain diseases/disorders. Along those lines, the current volume describes techniques for gene therapy involving endothelial nitric oxide synthase delivery to the lung in pulmonary hypertension and the techniques for delivery of the extracellular superoxide dismutase (ecSOD) gene for erectile dysfunction therapy. Drug delivery to the target areas has been identified as a preferred therapeutic approach to treat certain diseases, and this pharmacological strategy is being actively explored to combat certain oxidative stress-mediated diseases. In view of that, this volume describes the preparation of drug-loaded polymeric nanoparticles and evaluation of the antioxidant activity against lipid peroxidation, design, synthesis, and action of antiatherogenic antioxidants, synthesis and characterization of polymer nanocarriers for the targeted delivery of therapeutic enzymes *in vitro*, nanoparticle and iron

chelators as a potential novel Alzheimer therapy, and a simple method for effective and safe removal of membrane cholesterol from lipid rafts in endothelial cells with implications in the oxidant-mediated lipid signaling.

Molecular oxygen is the key life-supporting species in aerobes, and methods for the accurate measurement of oxygen levels in the biological systems are just emerging. For that, electron paramagnetic resonance spectroscopy (EPR) appears to be a novel analytical technique of choice, and the current volume presents the methods of EPR imaging of free radicals and oxygen in vivo and the EPR spectroscopic determination of tissue oxygen in vivo with the aid of oxygen-sensitive paramagnetic lithium phthalocyanine particles.

This book would not have been possible without the contribution of chapters by several experts in the field of oxidative stress, and therefore, the editors deem it a distinct privilege to gratefully acknowledge every individual author of the chapters that has made this book a reality. We thank Drs. Achuthan Raghavamenon, Michelle Fletcher Claville, and Deidra Atkins-Ball for help in editing and organizing the chapters. The patience shown by Professor Walker and the team of Humana Press is beyond words and cannot be adequately articulated. The editors express profound gratitude to Professor John Walker for the confidence shown and consider it an honor that he gave us this assignment. We also thank the following organizations and universities for financial support and use of institutional facilities: Departments of Chemistry and Environmental Toxicology, Southern University and A&M College, Baton Rouge, LA; Departments of Medicine and Pharmacology, Tulane University School of Medicine, New Orleans, LA; Department of Internal Medicine and the Davis Heart and Lung Research Institute, The Ohio State University Medical College, Columbus, OH; National Institutes of Health, Bethesda, MD; National Science Foundation, Washington, DC; and US Department of Education, Washington, DC. Finally, we would be remiss if we did not express our heartfelt appreciation to our families for their unparalleled support.

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