

METHODS IN MOLECULAR BIOLOGY

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Cryopreservation and Freeze-Drying Protocols

Third Edition

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Dedication

In memory of Stanley P. Leibo

Preface

Cryopreservation and freeze-drying are widely used for long-term storage of biological materials. Methods to safely store specimens in a stable state for extended periods have widespread applications in medicine and agriculture. Using cryopreservation for cell and tissue banking allows samples to be available at the time of need. Ice-free cryopreservation, referred to as vitrification, is receiving increased attention in biobanking. Whereas cryopreserved samples are typically stored in liquid nitrogen, dried specimens can be stored at room temperature, which has clear advantages for banking and transport. Freeze-drying involves a freezing and a drying step and hence requires protectants that protect during both freezing and drying.

A variety of biological materials can be cryopreserved using relatively easy and standard protocols. There are cases, however, which require custom-designed cryopreservation strategies including the use of nonconventional cryoprotective agents, optimized cooling and warming rates, selection and cleanup processing, and modification of cellular properties. Drying of cells or biomolecular assemblies is generally more damaging compared to cryopreservation, and requires addition of lyoprotectants. Freeze-drying is widely used to stabilize biomolecules and macromolecular assemblies and has been implicated as a method to preserve mammalian cells in a dry state. Cryopreservation and dry preservation are highly interdisciplinary fields of research requiring insights from biologists, chemists, physicists, as well as engineers to find rationally designed preservation solutions for individual cases.

In this edition of the book “Cryopreservation and Freeze-Drying Protocols” we not only aimed to provide a variety of standard protocols that can be used to cryopreserve or freeze-dry different types of specimens, but also wanted to highlight methods that can be used to obtain insights in cellular and macromolecular changes in response to freezing or drying that can be used to rationally design preservation protocols. The book is divided into four parts. Part I handles fundamental principles of cryopreservation, vitrification, freeze-drying, and the use of mathematical modeling to design preservation protocols. In Part II, microscopic, spectroscopic, as well as calorimetric methods are presented to study cell and molecular behavior during freezing and drying, as well as thermodynamic properties of preservation solutions. In Part III, cryopreservation and vitrification approaches are presented for a wide variety of samples including sperm, oocytes, blastocysts, mammalian and plant cell lines, stem cells, blood cells, and tissues. In addition, various preparative processing methods such as cleanup procedures and membrane modification strategies are presented. In Part IV, freeze-drying methods are described for proteins, bacteria, sperm, and extracellular tissue matrices.

The book aims to serve as a practical guideline that can be used without the need of other reference sources. In addition to protocols that rely on the use of specialized equipment, practical and cheaper alternatives are also described. Our intended readers are researchers and technical assistants in academia and industry with a background in life sciences or engineering who want to investigate freezing and drying processes or set up methods to safely store biological material while maintaining its function upon reconstitution.

Hannover, Germany
Hannover, Germany

Willem F. Wolkers
Harriëtte Oldenhof

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