

## Chapter 8.2

# Cryopreservation of Lymphocytes in Bulk

DOUGLAS M. STRONG

### Introduction

Since the early discovery of the cryoprotective properties of glycerol, a great deal of investigation has gone into the determination of the mechanisms of freezing injury (Polge et al., 1949). Very briefly, cells that are cooled too slowly, to below freezing temperatures, are damaged by the resulting increase in salt concentration and cellular shrinkage which occurs as water is removed during the formation of extracellular ice (Lovelock, 1953). Conversely, if cooling is too rapid, a new mechanism is invoked in which shrinkage no longer occurs but the cell is damaged by the formation of intracellular ice, either during freezing or upon thawing (Mazur et al., 1969). Cryoprotectants, such as  $\text{Me}_2\text{SO}$ , reduce the amount of ice present during freezing and reduce solute concentration thus reducing ionic stress (Farrant et al., 1974; Lovelock, 1953; Mazur et al., 1969; Polge et al., 1949). However, these compounds can themselves cause osmotic injury since they are hypertonic and can cause damage during their addition or removal (Strong, 1976). Optimum cooling rates vary from cell type to cell type depending on differences in membrane permeability and intracellular water, which is removed during the dehydration phase of slow cooling and extracellular ice formation. In general, the larger the cell volume, the slower the rate of cooling to allow equilibration of intra- and extra-cellular water during freezing.

Although the primary use of cryopreserved lymphocytes in the histocompatibility laboratory may be as reagents in lymphocytotoxicity assays, where the preservation requirements are less rigid, the use of frozen thawed cells in functional assays or in assays for determination of other cell surface markers, may require closer attention to protocol. Several laboratories have reported that, under certain conditions, selection of lymphoid subsets may occur following freezing and thawing (Farrant et al., 1972; Knight et al., 1972). Furthermore, lymphoid clones and B- or T-LCL have different optimum cooling rates (Strong et al., 1985). Although sophisticated controlled rate freezing devices are not absolutely required for lymphocyte cryopreservation, such equipment usually increases reproducibility and improves recovery (Dubey et al., 1986; Ichino and Ishiiwa, 1988). In addition, the handling of cells prior to and following freezing and thawing is at least as important as the freezing itself.

### Abbreviations

$\text{Me}_2\text{SO}$ : Dimethylsulfoxide  
DNase: Deoxyribonuclease

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**Tristram G. Parslow**

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## **Table of Contents Ashi Laboratory Manual Volume 2**

1. Understanding the eBook Ashi Laboratory Manual Volume 2
  - The Rise of Digital Reading Ashi Laboratory Manual Volume 2
  - Advantages of eBooks Over Traditional Books
2. Identifying Ashi Laboratory Manual Volume 2
  - Exploring Different Genres
  - Considering Fiction vs. Non-Fiction
  - Determining Your Reading Goals
3. Choosing the Right eBook Platform
  - Popular eBook Platforms
  - Features to Look for in an Ashi Laboratory Manual Volume 2
  - User-Friendly Interface
4. Exploring eBook Recommendations from Ashi Laboratory Manual Volume 2
  - Personalized Recommendations
  - Ashi Laboratory Manual Volume 2 User Reviews and Ratings
  - Ashi Laboratory Manual Volume 2 and Bestseller Lists
5. Accessing Ashi Laboratory Manual Volume 2 Free and Paid eBooks
  - Ashi Laboratory Manual Volume 2 Public Domain eBooks
  - Ashi Laboratory Manual Volume 2 eBook Subscription Services
  - Ashi Laboratory Manual Volume 2 Budget-Friendly Options

6. Navigating Ashi Laboratory Manual Volume 2 eBook Formats
  - ePUB, PDF, MOBI, and More
  - Ashi Laboratory Manual Volume 2 Compatibility with Devices
  - Ashi Laboratory Manual Volume 2 Enhanced eBook Features
7. Enhancing Your Reading Experience
  - Adjustable Fonts and Text Sizes of Ashi Laboratory Manual Volume 2
  - Highlighting and Note-Taking Ashi Laboratory Manual Volume 2
  - Interactive Elements Ashi Laboratory Manual Volume 2
8. Staying Engaged with Ashi Laboratory Manual Volume 2
  - Joining Online Reading Communities
  - Participating in Virtual Book Clubs
  - Following Authors and Publishers Ashi Laboratory Manual Volume 2
9. Balancing eBooks and Physical Books Ashi Laboratory Manual Volume 2
  - Benefits of a Digital Library
  - Creating a Diverse Reading Collection Ashi Laboratory Manual Volume 2
10. Overcoming Reading Challenges
  - Dealing with Digital Eye Strain
  - Minimizing Distractions
  - Managing Screen Time
11. Cultivating a Reading Routine Ashi Laboratory Manual Volume 2
  - Setting Reading Goals Ashi Laboratory Manual Volume 2
  - Carving Out Dedicated Reading Time
12. Sourcing Reliable Information of Ashi Laboratory Manual Volume 2
  - Fact-Checking eBook Content of Ashi Laboratory Manual Volume 2
  - Distinguishing Credible Sources
13. Promoting Lifelong Learning
  - Utilizing eBooks for Skill Development
  - Exploring Educational eBooks
14. Embracing eBook Trends
  - Integration of Multimedia Elements

- Interactive and Gamified eBooks

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