



Standard Practice for Preservation by Freezing, Freeze-Drying, and Low Temperature Maintenance of Bacteria, Fungi, Protista, Viruses, Genetic Elements, and Animal and Plant Tissues¹

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INTRODUCTION

Methods used for low temperature preservation of living biological systems include both freezing and freeze-drying. While in many cases other methods of preservation can be used, these low temperature methods provide the only real assurance of genetic stability. However, there are reports of damage to DNA as a result of freeze-drying **(1)**.²

This practice assumes a basic knowledge of freezing and freeze-drying methods, and does not include specific methods used for freezing and freeze-drying.

1. Scope

1.1 This practice covers the handling of microorganisms (bacteria, fungi, and protista), viruses, genetic elements (nucleic acids and plasmids), and animal and plant cell tissues (cell lines), during and after freezing and storage at cryogenic temperatures.

1.2 This practice also covers the handling of microorganisms, viruses, and genetic elements in the host cell during and after freeze-drying.

1.3 While this practice does not cover the specific methodology used to freeze and freeze-dry microorganisms and cell lines, the safety aspects of handling microorganisms during freezing and freeze-drying procedures, and during storage at cryogenic temperatures, are covered. Other guidelines must also be adhered to regarding the handling of hazardous materials **(2)**.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* For specific hazard statements see Section 6.

2. Referenced Documents

2.1 ASTM Standards:

E 1564 Guide for Design and Maintenance of Low-Temperature Storage Facilities for Maintaining Cryopreserved Biological Materials³

E 1565 Guide for Inventory Control and Handling of Biological Materials Maintained at Low Temperatures³

E 1566 Guide for Handling Hazardous Biological Materials in Liquid Nitrogen³

3. Terminology

3.1 Definitions of Terms Specific to This Standard:

3.1.1 *cryogenic temperatures*—temperatures below or equal to -100°C .

3.1.2 *cryoprotectant*—a chemical substance used to protect cells during freezing and rewarming.

3.1.3 *eutectic temperature*—the temperature below which all liquid portions of an aqueous suspension have entered the solid phase.

3.1.4 *freeze-drying*—sublimation of water from a frozen aqueous suspension.

3.1.5 *freezing*—lowering the temperature of an aqueous suspension to a point at or below the temperature of ice crystal formation.

3.1.6 *low temperature preservation*—stabilizing viable or biologically active material by freezing or freeze-drying.

3.1.7 *vitrification*—solidification of an aqueous suspension at low temperatures without the formation of ice crystals.

4. Significance and Use

4.1 The stability of cell populations and genetic elements at low temperatures can be affected by the methods used to

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² The boldface numbers in parentheses refer to the list of references at the end of this practice.

³ *Annual Book of ASTM Standards*, Vol 11.05.