



StorZ™ Cryopreservation Medium (with DMSO¹)



Product Name: StorZ™ CRYOPRESERVATION MEDIUM

Product Codes: STZ-100, STZ-500

General Use: Cryopreservation of Cells and Tissues from Humans and Other Mammals

Features: GMP quality; sterile; USP grade materials; no animal components; no human proteins; no antibiotics

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General Description

StorZ™ (with DMSO) and its non-DMSO counterpart, StorZ-ND™, are members of the “EZ-CPZ™ Family” of proprietary, chemically defined, GMP-manufactured clinical grade cryomedia for cryopreservation or cold storage of human cells and tissues (Table 1). StorZ™ is a “1X, ready-to-use” solution for tissue storage or to resuspend and store cells after concentration by centrifugation or other methods.

StorZ™ is derived from a “universal” cryo-media formula, EZ-CPZ™, which has been used with a variety of cell and tissue types (Table 2). Although the indicated tissues and cell types have been stored in “StorZ-like” media, they have not all been specifically tested directly with StorZ™. StorZ™ may be used for ultracold cryopreservation and storage of cells in the vapor phase of liquid nitrogen, and for tissues frozen at ≤(-)80°C storage or refrigerated (2°C to 8°C) storage.

Formulation and Safety

StorZ™ contains 5% DMSO. StorZ™ and other EZ-CPZ™ family cryomedia components are high quality USP or pharmaceutical grade chemicals (including trehalose, other sugars, salts and amino acids) from plant and microbial sources. The chemicals are thus considered “xeno-free” in not being derived from animal sources, such as blood products or other proteins. In pre-clinical studies, the cryomedia has been non-toxic when injected into animals by various routes. The media products are currently included in ongoing clinical trials with no adverse reaction or safety concerns reported.

Use and Methods

Cells cryostored in StorZ™ retain high viability and excellent growth and differentiation potential when re-animated. StorZ™ can be used with cells that were grown in serum-free, defined media, in standard media with sera, or in animal component-free media. Stored cells have been successfully reanimated with very high recovery, viability, growth and various cell-specific differentiation capabilities. Sources would include freshly isolated tissues and cells, in vitro cultured cells, and established cell lines (as per the original “parent 2X media” EZ-CPZ™ or EZ-CPZ-ND™ formulas; Tables 1 and 2).

Clinical Tissue/Cell Specimens and Early Passage Cultured Cells

Storage. Freshly obtained tissues or cells can be derived and stored from various types of human solid tissues, blood cells, and tumor cells (Table 2). Comparable results can be obtained from similar mammalian tissues. Cells removed from adherent cultures or from suspension cultures are concentrated by centrifugation, counted and resuspended to a known density of cells/mL (depending on cell type), or other preferred cell density not to exceed 20% of the total volume of cryopreservation medium. Addition of a small percentage of human serum or human serum albumin is optional and may improve post-storage recovery of certain cells, but is not recommended if the goal is to maintain a chemically defined system. Cryovials or bags are placed in a controlled rate freezer container according to the user’s freezing protocol, or in an insulated container (e.g., “Mr. Frosty”) with isopropanol at -80°C for at least 12 hours to cool down at about 1 degree per minute. It is then transferred to an ultralow ≤-135°C freezer or liquid nitrogen vapor phase freezer for storage.

Recovery and Reanimation. Quick-thaw, rapid recovery of frozen cells is done by placing cryovials in a 37°C water bath for ~1 min until the storage medium has thawed. Cells are immediately placed in a pre-warmed cell culture media (<5 min from thaw). Cells are extremely fragile at this time, so handle gently. Most cells recover from cryopreservation after they are grown for a few

Table 1. EZ-CPZ™ Media Family, Including StorZ™ Media: Designations and Descriptions

Media Designation	Product Code	Concentrations Formula (X); %DMSO	Cell Types
EZ-CPZ™	EZCN	2X; 10% DMSO	All
EZ-CPZ-ND™	EZCND	2X; no DMSO	All
StorZ™ Media (1X Formulations of EZ-CPZ™)			
StorZ™	STZ	1X; 5% DMSO	All
StorZ-ND™	STZND	1X; no DMSO	All
StorZ-B™	STZB	1X; 5% DMSO	Blood
StorZ-BND™	STZBND	1X; no DMSO	Blood

Legend. The EZ-CPZ™ media are generally used as “2X” media mixed 1:1 with cell suspensions in their preferred media. StorZ™ media are “1X” EZ-CPZ™ or EZ-CPZ-ND™ derived solutions used to re-suspend cell concentrates prior to cryostorage. The “ND (No DMSO) media can have DMSO or other cryoprotectants added at preferred concentrations. “B” designates formulas for blood cells and cultured blood cell lines.

¹ DMSO: Dimethyl Sulfoxide

hours or overnight before replacing the medium. Some cells are centrifuged to remove the cryoprotectant media and then gently re-suspended in growth media. Tissues are rinsed upon recovery before use. Cells recovered from these sources and initiated in culture post cryostorage may have few or no subcultures, and have a range of recovered viability from a low of 75% to high percentages of 90% or greater in trypan blue dye exclusion or other viability assays. Note: Non-DMSO formulas may have only 60% to 80% viable cells and/or delayed growth at recovery.

Manufacturing

Media are manufactured by sterile 0.22 µm filtration and packaging, using cGMP standards in an ISO Class 7 clean room and ISO Class 5 biosafety cabinet. Raw materials are pre-tested and the final product is checked for endotoxin, sterility (bacteria, fungi, and mycoplasma) and other quality specifications and criteria (see below) prior to release and preparation of the Certificate of Analysis.

Specifications

Visual clarity
pH (USP <791>)
Osmolality (USP <785>)
Sterility: SC (USP <71>)
Sterility: FTG (USP <71>)
Mycoplasma (USP <63>)
Endotoxin (USP <85>)
Expiration

Acceptance Criteria

Clear, colorless
6.8 to 7.5
800 to 1400 mmol/kg
No microbial growth
No microbial growth
None detected
<0.5 EU/mL
18 months from date of manufacture

Storage

Store media refrigerated at 2°C to 8°C. Do not store frozen. StorZ™ must be stored in a light-protective bag or other suitable container to protect from light.

Master Files Applications Note

StorZ™ is a member of the EZ-CPZ™ media family, which is in FDA Drug and Device Master Files. These media have not been tested by INCELL for any specific diagnostic or therapeutic use. To request use of a Master File call, fax, or email to masterfiles@incell.com.



Animal Component Free. INCELL certifies that these media have no “animal-derived components” per the following criteria:

- No animal-derived components are added by INCELL or come from raw materials supplied as components of the Product.
- Product does not come into contact with animal-derived material during manufacturing, processing, handling, or packaging.
- Product manufacturing does not include shared equipment exposed to animal-derived components.
- This certification applies only to the condition of the above-described Product in its unopened package, and INCELL assumes no responsibility for a Product failing to meet this Statement after handling or use after opening the package.
- Signed “Animal Origin Statement” can be provided on request.

Ordering: Contact Charter Medical

Toll Free: 866.458.3116
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Technical Assistance

The scientists at INCELL are available to discuss the media or special needs of your cells, and to assist you in your cell culture applications. Call 1-800-364-1765 or e-mail info@incell.com.

Supportive Data on “EZ-CPZ Family” Media Use

The following uses of EZ-CPZ™ in 1:1 mix with various cell-preferred media, with or without supplements, including sera.

Table 2. Examples of Human Cells Stored in “Parental” Media EZ-CPZ™ and/or EZ-CPZ-ND™ for Various Applications

Cells for Regenerative Medicine	
Human Tissues and Cell Sources	Primary Cell Type(s)
Adipose (Fat)	Mesenchymal Stem Cells; Stromal vascular fraction regenerative cells
Bone Marrow; Bone, Cartilage, Adipocytes	Hematopoietic and mesenchymal stem cells; various types of renewable progenitor cells; Endothelial cells
Nucleus pulposus (NP) Intervertebral Disc	NP stem cells; annulus chondrocytes and mesenchymal stem cells; various renewable progenitor cells
Parathyroid	Adenoma cells isolated and stored for potential re-transplantation
Peripheral or apheresis blood	Various white blood cells, e.g., lymphocytes, macrophages, etc.; Circulating, mesenchymal cells; endothelial cells.
Placenta	Trophoblasts; Syncytiotrophoblasts; Endothelial cells; Hematopoietic; mesenchymal stem cells; various progenitors
Skin (adult; foreskin)	Epidermal keratinocytes; biopsies; Dermal fibroblasts; mesenchymal cells
Tumors	Epithelial, mesenchymal, lymphoid
Umbilical cord	Hematopoietic and mesenchymal stem cells; various types of renewable progenitor cells; Endothelial cells
Cell Lines	CHO, HEK, BHK, L929, HUVEC, COS, 293, 3T3, U937, HT29, HL60, and INCELL's proprietary cell lines NCM460D™, NCM356D™, IBHK-4, and E10 (anti-HHV-8) hybridoma