# EZ-CPZ™ Cryopreservation Medium (with DMSO[1])

**Product Name:** EZ-CPZ™ Cryopreservation Medium (with DMSO)

**Product Codes:** EZCN-100

**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

EZ-CPZ™ is a universal GMP-manufactured family of media (Table 1) for cryopreservation or cold storage of human cells and tissues intended for clinical use or for special research applications. It is a proprietary, chemically-defined formula of clinical grade components, including 10% (v/v) dimethyl sulfoxide (DMSO) cryoprotectant. EZ-CPZ™, and its DMSO-free counterpart, EZ-CPZ-ND™ are packaged in a convenient, 2X formula. For cells, they are generally used as a 2X formula mixed 1:1 with cells suspended to a known number in their preferred growth or conditioned medium. This results in a final DMSO concentration of 5%, which is the concentration in a "1X" derived media mixture used to resuspend concentrated cells for cryostorage in the vapor phase of liquid nitrogen, and for tissues frozen at ≤(-)80°C storage or refrigerated (2°C to 8°C) storage. For some tissues or cells, EZ-CPZ™ or EZ-CPZ-ND™ is used undiluted or at other dilution ratios. All media can have additional supplements, such as serum, added.

**Table 1. EZ-CPZ™ Media Family 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 |

Legend. The EZ-CPZ™ media are generally used as "2X" media mixed 1:1 with cell suspensions in their preferred media to be "1X' for use.

## Formulation and Safety

The EZ-CPZ™ 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 components, 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. When used in a 1:1 ratio of EZ-CPZ™ mixed with media or cells suspended in media, the final DMSO concentration is 5%.

## Use and Methods

EZ-CPZ™, a "high performance" cryomedia for many types of human and animal cells and tissues, has been used with freshly isolated tissues and cells, in vitro cultured cells, and cell lines (Tables 2, 3). Stored cells have been successfully reanimated with very high recovery, viability, growth and various cell-specific differentiation capabilities. EZ-CPZ™ generally results in 70% to 90% or more recovery of viable cells and protects cultured cells in their growth recovery but use with specific cells, tissues or cell lines needs to be tested. Recommended use is a 1:1 mix of cells in their preferred culture or conditioned media and EZ-CPZ™. ND media can have DMSO or other cryoprotectants added.

**Table 2. Human Cells Stored in EZ-CPZ ™ and/or EZ-CPZ-ND™ for Various Biomedical, Clinical and Research Applications**

| Tissues and Cells for Regenerative Medicine | |
|---|---|
| **Human Tissues** | **Primary Cell Type(s)** |
| Adipose (Fat) | Mesenchymal Stem Cells; Stromal vascular fraction 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 or mesenchymal cells; endothelial cells. |
| Placenta | Trophoblasts; syncytiotrophoblasts; endothelial, hematopoietic and mesenchymal stem cells; various renewable progenitor cells |
| 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; INCELL's proprietary cell lines NCM460D™, NCM356D™, IBHK-4, and E10 (anti-HHV-8) hybridoma |

[1] DMSO: Dimethyl Sulfoxide

## Clinical Tissue/Cell Specimens and Early Passage Cells

**Storage.** Freshly obtained tissues or cells can be derived and stored from various types of human and animal solid tissues, blood cells, and tumor cells (Tables 2 and 3). 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 ~1 degree per minute. It is then transferred to an ultralow ≤(-)135°C freezer or liquid nitrogen vapor phase 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 to 5 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 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 i.e., low of 70% to high 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.

**Table 3. Animal Tissues and Cells Stored in EZ-CPZ ™ and/or EZ-CPZ-ND™ for Various Biomedical Applications**

| Cells and Tissues Derived from Adult, Newborn and/or Fetal Sources | |
|---|---|
| **Animal Tissues** | **Cultured Cell Types** |
| Adipose (Fat) | Mesenchymal Stem Cells; Stromal vascular fraction cells; adipose cells |
| Bone Marrow | Hematopoietic and mesenchymal stem cells; various progenitor cells; endothelial cells; many cell subsets |
| Brain /Neural (Spinal) | Progenitors; Induction of differentiation |
| Colon; Stomach; Gastrointestinal | Primary epithelial and/or mesenchymal support cells; complex organ-like cultures |
| Kidney | Primary epithelial cells and/or mesenchymal support cells |
| Liver | Primary epithelial cells and/or mesenchymal support cells |
| Muscles (Heart; Smooth; Peripheral;) | Pericytes; Mesenchymal or Stromal Stem Cells; other regenerative cells |
| Pancreas; Other Endocrine organs | Pancreatic islet beta and acinar cells; other organs (*e.g.,* adrenal) |
| Peripheral blood | Circulating or mesenchymal cells; endothelial and blood cells |
| Skin (adult; newborn) | Epidermal keratinocytes co-cultures; Dermal; other mesenchymal cells; |
| Spleen | Endothelial and blood cells; lymphocytes |
| Tumors; various | Epithelial, mesenchymal, lymphoid; others |

Legend. Various cell types included INCELL's M3™, M3Z™ clinical use media or other standard, commercially media.

**EZ-CPZ™**
Cryopreservation Medium


## Manufacturing

EZ-CPZ media is 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 by quality specifications and acceptance criteria per USP standards for pH, osmolality, sterility (bacteria, fungi), mycoplasma and endotoxin prior to release and preparation of a Certificate of Analysis.

| Test Methods | Acceptance Criteria |
|---|---|
| Visual clarity | Clear, colorless |
| pH (USP <791>) | 6.8 to 7.5 |
| Osmolality (USP <785>) | 320 to 530 mmol/kg (1:5 dilution) |
| Sterility: SC (USP <71>) | No microbial growth |
| Sterility: fTG (USP <71>) | No microbial growth |
| Mycoplasma (USP <63>) | None detected |
| Endotoxin (USP <85>) | <0.5 EU/mL |

## Storage

EZ-CPZ™ is stored in amber bottles or in a light-protective bag, or other suitable container, at 2°C to 8°C. Do not store frozen. Shelf-life is 18 months from date of manufacture.

## Master Files Applications Note

The EZ-CPZ™ media family is in FDA Drug and Device Master Files but 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 info@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 INCELL Corporation

Toll Free: 800.364.1765
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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 800.364.1765 or e-mail info@incell.com.