



## EZ-CPZ-ND™ Cryopreservation Medium (No DMSO<sup>1</sup>)



**Product Name:** EZ-CPZ-ND™ Cryopreservation Medium (No DMSO)

**Product Codes:** EZND-020, EZND-100, EZND-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

EZ-CPZ-ND™ and its DMSO-containing counterpart, EZ-CPZ™, are proprietary, chemically defined, GMP-manufactured EZ-CPZ™ media family cryomedia (Table 1) for cryopreservation or cold storage of human cells and tissues. The formulas have clinical grade components and cryo-protective water binding and vitrification agents and EZ-CPZ-ND™ contains no DMSO. The cryostorage media are in a ready-to-use formula intended for clinical use or for special research applications. For cells, they are generally used as a “2X” formula mixed 1:1 with cells suspended in their preferred growth or conditioned medium. This results in a final DMSO concentration of 5%, which is the concentration in the “1X” derived media mix 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. 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

EZ-CPZ-ND™ 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. When used in a 1:1 ratio of EZ-CPZ™ mixed with media or cells suspended in media, the final DMSO concentration is 5%. Users can optionally custom formulate EZ-CPZ-ND™ for cell type optimization with certain cryoprotectants or other additives, including DMSO, at low concentrations.

### Use and Methods

EZ-CPZ-ND™ is a “high performance” concentrated media that 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. For most cells EZ-CPZ-ND™ has 60% to 70% viability (compared to EZ-CPZ™ (70% to 90%) viability), and delayed growth recovery but comparable growth rates and cell densities upon recovery. Use is recommended as a 1:1 mixture with suspended cells and with optional addition of cryoprotectants at preferred concentrations.

**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

<sup>1</sup> DMSO: Dimethyl Sulfoxide

## Clinical Tissue/Cell Specimens and Cultured 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  $\leq(-)135^{\circ}\text{C}$  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. Tissues are rinsed upon recovery before use. EZ-CPZ-ND™ may have only 60% to 80% viable cells at recovery, i.e., lower than EZ-CPZ™ (70% to 90%).

## Manufacturing

EZ-CPZ-ND 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 tested by USP standards for pH, osmolality, sterility (bacteria, fungi), mycoplasma and endotoxin 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>)

## Acceptance Criteria

Clear, colorless  
6.8 to 7.5  
600 to 1000 mmol/kg  
No microbial growth  
No microbial growth  
None detected  
<0.5 EU/mL

## Storage of EZ-CPZ-ND™

Store media refrigerated at 2°C to 8°C. Do not store frozen. Shelf-life is 18 months from date of manufacture.

## Master Files Applications Note

EZ-CPZ-ND™ and EZ-CPZ™ media are 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](mailto: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  
[info@incell.com](mailto:info@incell.com)  
FAX: 210.877.0200

## 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](mailto:info@incell.com).

**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-ND™

### Cryopreservation Medium

