

# Specification Sheet

Product Name	CRYO-JIN™, liquid, sterile-filtered, 100 mL
Product Number	10004-01
Product Brand	Revive Organtech, Inc
Storage Temp	2-8°C
<b>TEST</b>	<b>SPECIFICATION</b>
Appearance (Turbidity)	Clear, Yellow Color
Appearance (Form)	Solution
pH	7.80~8.20
Sterility	Sterile Filtered (0.22uM)
Endotoxin Level	N/A
<b>Instructions for use</b>	<p>CRYO-JIN™ is a premium <b>primary hepatocyte cryopreservation medium</b>. Designed to prepare and preserve hepatocyte in ultralow temperature environments (-80 to -196 °C), CRYO-JIN™ provides a safe, protective environment for hepatocyte during the freezing, storage, and thawing process. CRYO-JIN™ contained proprietary components which are directly reducing the level of freezing induced apoptosis and necrosis and improving post-thaw hepatocyte viability and function. CRYO-JIN™ is recommended for the preservation of <b>primary hepatocyte</b>. CRYO-JIN™ is cGMP-mimic condition manufactured with high quality grade components.</p> <p><b>Preparation instructions</b></p> <p>The CRYO-JIN™ solution is ready-to-use and complete with no additives required. Wipe down the outside of container with 70% alcohol before opening as the contents are sterile. If the seal has been broken, do not use.</p> <p><b>Storage/Stability</b></p> <p>Store the CRYO-JIN™ solution at 2-8°C and protected from light until ready to use.</p> <p><b>Freezing Procedures for Cells</b></p> <ol style="list-style-type: none"> <li>1. Suspended cell to be cryopreserved using mechanically or enzymatically dissociation.</li> <li>2. Centrifuge the cells to obtained pellet.</li> <li>3. Remove supernatant (remove the culture media as possible to reduce dilution of the CRYO-JIN™ solution).</li> </ol>

4. Add Ambient temperature CRYO-JIN™ solution to a cell concentration range of  $1-10 \times 10^6$  cells/mL for standard cell culture protocol.
5. After mixed with CR CRYO-JIN™ solution with cells, incubated for 10 minutes at 1~4°C to penetrate cryoprotectants inside of **cells (in case of small tissue or organoid, 20 minutes incubation at 1~4°C)**
6. Nucleation-lower sample temperature -80°C; After cells are mixed with solution, put cryovial into controlled rate freezer (-1°C/minute) and then freeze to -80°C (slow freezing method), or put cryovial into Bicell, Mr. Frosty™ Freezing container, or similar kinds of slow freezing container and put the cryovial included in such slow freezing container into -80 °C freezer.

#### **Storage procedures for cells**

1. After finished the nucleation of cryo samples at -80°C freezer, store the freeze samples at liquid nitrogen tank for long term storage (below -130°C).

#### **Thawing procedures for cells**

1. Thawing the freeze hepatocyte quickly at 37°C water bath. hepatocyte should be thawed with gentle swirling of the hepatocyte until all visible ice has melted.
2. Dilute cell/CRYO-JIN™ mixture immediately with appropriate thawing medium. The dilution thawing medium should be 20~37°C. **A dilution ratio of 1:50 (sample: thawing medium) is recommend.** After diluting cells with warmed thawing medium, gently inverted for 5~10 times and follow by centrifuge (Highly recommend at 50G for 5 minutes).
3. After centrifuge, completely suction of supernatant and add new warmed culture media.
4. Plate the cells appropriately and cultured the cells or use immediately.