

# Specification Sheet

Product Name	CRYO-GOLD™, liquid, sterile-filtered, 100 mL
Product Number	10003-01
Product Brand	Revive Organtech, Inc
Storage Temp	2-8°C
<b>TEST</b>	<b>SPECIFICATION</b>
Appearance (Turbidity)	Clear
Appearance (Form)	Solution
pH	7.80~8.20
Sterility	Sterile Filtered (0.22uM)
Endotoxin Level	N/A
<b>Instructions for use</b>	<p>CRYO-GOLD™ is a serum-free, protein free, animal origin-free, and fully defined cryopreservation medium. Designed to prepare and preserve cells in ultralow temperature environments (-80 to -196°C), CRYO-GOLD™ provides a safe, protective environment for cells and tissues during the freezing, storage, and thawing process. CRYO-GOLD™ contained proprietary components which are directly reducing the level of freezing induced apoptosis and necrosis and improving post-thaw cell viability and function. CRYO-GOLD™ is recommended for the preservation of any types of cell lines (CHO, VERO, MDCK, MCF-7, Caco2, HEK293, etc.), bone marrow, epithelial cells, endothelial cell, human fibroblast, keratinocyte, cord blood cells, PBMC, adult stem cell (MSCs), embryonic and induced pluripotent stem cells (ESCs and iPSCs), other extremely sensitive cell types, and small size of tissues. CRYO-GOLD™ is cGMP-mimic condition manufactured with high quality grade components.</p> <p><b>Preparation instructions</b></p> <p>The CRYO-GOLD™ 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 it.</p> <p><b>Storage/Stability</b></p> <p>Store the CRYO-GOLD™ solution at 2-8°C and protected from light until ready to use.</p>

### Freezing Procedures

1. Suspended cell to be cryopreserved using mechanically or enzymatically dissociation.
2. Centrifuge the cells to obtained pellet.
3. Remove supernatant (remove the culture media as possible to reduce dilution of the CRYO-GOLD™ solution).
4. Add Ambient temperature CRYO-GOLD™ solution to a cell concentration range of  $1-10 \times 10^6$  cells / 1 ml of CRYO-GOLD™ for standard cell culture protocol.
5. After mixed with CRYO-GOLD™ 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

1. After finished the nucleation of cells at -80°C freezer or controlled rate freezer, store the freeze cell at liquid nitrogen tank (below -130°C).

### Thawing Procedures

1. Thaw samples quickly in a 37°C water bath. Samples should be thawed with gentle swirling of the sample until all visible ice has melted (*Do not allow sample to warm above chilled temperatures(0-10°C). Cryovials should be cool to the touch when removed from the water bath.*)
2. Dilute cell/CRYO-GOLD™ mixture immediately with appropriate culture medium. The dilution culture medium should be 20~37°C. A dilution ratio of 1:10 (sample: culture medium) or greater is recommend. After diluting cells with warmed culture medium, gently inverted for 5~10 times and follow by centrifuge (recommend at 1000 rpm for 5 minutes).
3. After centrifuge, completely suction of supernatant and add new warmed culture media.
4. Plate the cells appropriately and culture the cells or use immediately.