REVIVE ORGANTECH, Inc
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Specification Sheet

Product Name	CRYO-SOL™, liquid, sterile-filtered, 100 mL
Product Number	10007-01
Product Brand	Revive Organtech, Inc
Storage Temp	2-8°C
TEST	**SPECIFICATION**
Appearance (Turbidity)	Clear Color
Appearance (Form)	Solution
pH	7.80~8.20
Sterility	Sterile Filtered (0.22uM)
Endotoxin Level	N/A
Instructions for use	CRYO-SOL™ is a very high-quality freezing media for extremely cryopreservation difficult samples such as primary human/animal hepatocytes, 3D engineered tissues, small tissue, spheroids, or organoids. Designed to prepare and preserve extremely cryopreservation difficult samples such as primary human/animal hepatocytes, 3D engineered tissues, small tissue, spheroids, or organoids in ultralow temperature environments (80 to -196 °C). CRYO-SOL™ provides a safe, protective environment for extremely cryopreservation difficult such as primary human/animal hepatocytes, 3D engineered tissues, small tissue, spheroids, or organoids during the freezing, storage, and thawing process. CRYO-SOL™ contained proprietary components which are directly reducing ice crystal formation during the freezing and thawing stage. CRYO-SOL™ contained components which are directly reducing level of freezing induced apoptosis and necrosis and improving post-thaw extremely cryopreservation difficult such as primary human/animal hepatocytes, 3D engineered tissues, small tissue, spheroids, or organoid's viability and functionality. CRYO-SOL™ is recommended for the preservation of primary human/animal hepatocytes, 3D engineered tissues, small tissue, spheroids, or organoids. CRYO-SOL™ is cGMP-mimic condition manufactured with high quality grade components. **Preparation instructions** The CRYO-SOL™ 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. **Storage/Stability** Store the CRYO-SOL™ solution at 2-8ºC until ready to use.

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Freezing Procedures for samples

1. Suspend 3D engineered tissues, small tissues, spheroids, or organoids in culture media or washing media.
2. Centrifuge the samples to obtain pellets.

3. Suction of supernatant (completely remove the supernatant to reduce dilution of the CRYO-SOL™ solution's composition). *If supernatant remains, it will break the CRYO-SOL compositions, So, we recommend **2 suction cycles**. First, suction and place it on that conical tube a lack, after 30 seconds, using a 200 uL pipette tip, carefully remove the remaining supernatant completely.

4. Resuspend the cold (2-8ºC) CRYO-SOL™ solution with spheroids, or organoids, concentration range of 1,000~1,500 organoids or spheroids **(at least 1.5 ml of CRYO-SOL must be used for each vial).** It is never allowed to use less than 1 mM of freezing media for each cell vials. Due to the high viscosity of CRYO-SOL, we recommend gentle pipetting for mixing spheroids or organoids with CRYO-SOL. If you perform the quick or harsh pipetting with spheroids or organoids during the procedure, physical damage of spheroids or organoids will be occurring.

5. To completely penetrate small tissues, spheroids, or organoid samples with the cryoprotectant, incubate them for 30 minutes at 0–1ºC (ice chamber). During the cold incubation, cryovial has to be gently inverted several times each 5 minutes (total: 6 times).

6. Nucleation lower sample temperature -80ºC; Put cryovial into controlled rate freezer (-1ºC/minute) and then freeze to -80ºC, or put cryovial into Bicell, Mr. Frosty™ Freezing container, or similar kinds of slow freezing container and put into -80 ºC freezer.

Storage procedures for cells

1. After finishing the nucleation of cryo samples at-80ºC freezer, store the freeze samples at -80ºC or move them to the liquid nitrogen tank for long term storage (below -130ºC).

Thawing procedures for cells

1. Thawing the freezing samples quickly at 37ºC water bath. Cryovial should be thawed with gentle swirling until almost visible ice Melted)

2. Dilute the Samples/CRYO-SOL™ mixture immediately with appropriate thawing medium (Highly recommend using ThermoFisher thawing media (cat: CM7500). If that is unavailable, **we recommend making a thawing medium, such as organoid culture medium + 5% D-(+)-Glucose + 10μM dexamethasone).**

3. The dilution thawing medium should be 20~37ºC. **A dilution ratio of 1:8(sample: thawing medium) is highly recommended (prepare the 12 mL thawing media).** After diluting samples with warmed thawing medium, gently inverted for 5~10 times and follow by centrifuge (1000 rpm for 5 mins at Room Temperature or follow by user manual).

4. After centrifuge, completely suction of supernatant and add new warmed culture media.

5. Plate samples appropriately and culture the samples for use immediately.