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REVIVE ORGANTECH, Inc
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Specification Sheet

Product Name	**CRYO-DMSO-F**, liquid, sterile-filtered, 100 mL
Product Number	10005-01
Product Brand	Revive Organtech, Inc
Storage Temp	2-8°C
TEST	**SPECIFICATION**
Appearance (Turbidity)	Clear liquid
Appearance (Form)	Solution
pH	7.80~8.20
Sterility	Sterile Filtered (0.22uM)
Endotoxin Level	N/A
Instructions for use	CRYO-DMSO-F is a serum-free, protein free, animal origin-free, **DMSO Free** and fully defined cryopreservation medium. Designed to prepare and preserve cells in ultralow temperature environments (-80 to -196°C), CRYO-DMSO-F provides a safe, protective environment for cells during the freezing, storage, and thawing process. CRYO-DMSO-F contained proprietary components which are directly reducing the level of freezing induced apoptosis and necrosis and improving post-thaw cell viability and function. CRYO-DMSO-F is recommended for the preservation of Primary cells, iPSC, MS, MSC, Oocyte, Embryo, CAR-T, NK, and Macrophage cells. CRYO-DMSO-F is cGMP-mimic condition manufactured with high quality grade components. **Preparation instructions** The CRYO-DMSO-F 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-DMSO-F solution at 2-8ºC and protected from light until ready to use it. **Freezing Procedures** 1. Suspended cell to be cryopreserved using mechanically or enzymatically dissociation.

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2. Centrifuge the cells to obtained pellet.

3. Remove supernatant (remove the culture media as possible to reduce dilution of the CRYO-DMSO-F solution).

4. Add Ambient temperature CRYO-DMSO-F solution to a cell concentration range of 1-10×10^7 cells / 1 ml of CRYO-DMSO-F for standard cell culture protocol.

5. After mixed with CRYO-DMSO-F solution with cells, incubated for 10 minutes at 1~4ºC to penetrate cryoprotectants inside of **cells (in case of small tissue or organoid, 20 minutes incubation at 1~4ºC)**

6. Nucleation-lower sample temperature -80ºC; After cells are mixed with solution, put cryovial into controlled rate freezer (-1ºC/minute) and then freeze to -80ºC (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 ºC freezer.

Storage

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

Thawing Procedures

1. Thaw samples quickly in a 37ºC 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(0-10ºC). Cryovials should be cool to the touch when removed from the water bath).*

2. Dilute cell/CRYO-DMSO-F mixture immediately with appropriate culture medium. Add 20 ml of culture medium to 50 ml conical tube and gently mix with thawed cell/CRYO-DMSO-F mixture to culture medium (The dilution culture medium should be 20~37ºC). **A dilution ratio of 1:20 V: V (1 mL of thaw cell + 20 ml of culture media)** or greater is recommended. After diluting cells with warmed culture medium, gently inverted for 5~10 times and follow by centrifuge (recommend at 400G 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.