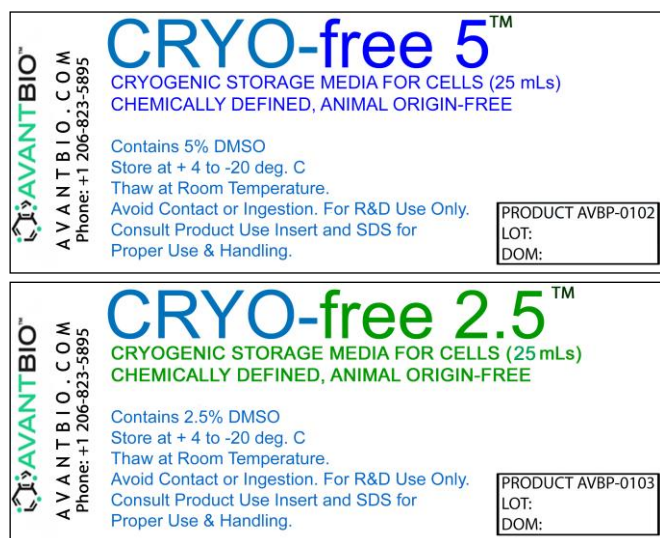




CRYO-free™ 5% or 2.5% DMSO

STERILE, CHEMICALLY DEFINED, ANIMAL ORIGIN-FREE (cdAOF) CELL CRYOPRESERVATION MEDIUM

25 mL Liquid Per Bottle. Contains 5 or 2.5 percent DMSO. Product number: AVBP-0102 (5% DMSO) and Product number: AVBP-0103 (2.5% DMSO), respectively. **Customer Support: Call +1 (206) 823-5895, email info@avantbio.com or visit www.avantbio.com.**

PRODUCT DESCRIPTION

CRYO-free™ is a physiologically-buffered, sterile, defined, animal origin-free, serum-free liquid cryopreservation medium containing 5% or 2.5% dimethylsulfoxide (DMSO). CRYO-free™ contains only USP-certified and other high-quality ingredients. CRYO-free™ does not contain undefined products, or any animal or human-derived products.

INTENDED USE

CRYO-free™ is intended for low temperature freezing and storage of mammalian cells or tissue in the liquid nitrogen (LN₂) vapor-phase (-196 °C). **This product is for laboratory research use only and is not intended for use in animals, humans, or for use in diagnostic procedures. CAUTION: CONTAINS DMSO.** Take appropriate precautions regarding the proper use and disposal of DMSO containing solutions. When handling this product, wear of protective clothing and eye wear. Dispose of CRYO-free™ properly.

STORAGE AND STABILITY

CRYO-free™ should be stored at -20 °C and thawed before use. Upon thawing CRYO-free should be

mixed by swirling the bottle, and then stored at 2-8 °C, in the dark thereafter. CRYO-free™ should not be freeze thawed. Protect CRYO-free™ from excessive heat and UV or visible light. CRYO-free is guaranteed for 6 months at 2-8 °C and 18 months at -20 °C.

INSTRUCTIONS FOR USE

1. Store CRYO-free™ at 2-8 °C until it is ready for use. To help maintain sterility, wipe down outside of bottle in a sterile field with 70% ethanol before removing the lid. The contents are sterile. If the seal has been broken or tampered, do not use. Gently mix (swirl) CRYO-free™ medium prior to use.
2. If your cells are adherent, enzymatically remove the cells from their subculture surface using dAOF cell dissociation reagents (TrypLE™ Select, Life Technologies cat. no. 12563-029). Collect and pellet the cells using centrifugation. After removing the supernatant, resuspend the cell pellet in cold (2-8 °C) CRYO-free™ medium and store cells temporarily at 2-8 °C (e.g., on ice). If required, rapidly count viable and non-viable cells, and adjust volume to obtain the desired cell concentration, typically 4 x 10⁵ to 4 x 10⁶ cells/mL.
3. Quickly distribute the cell suspension (typically 0.5-2 mL) into cooled cryopreservation vials and immediately cool each vial of cells to 2-8 °C.
4. Cryopreserve the cells by controlled temperature reduction (descent) to the LN₂ vapor-phase temperature. It is recommended that you follow a standard slow-rate (e.g., 1 °C/min), controlled cooling protocol to the LN₂ vapor-phase temperature. If precise controlled rate cooling is not possible in your laboratory, the cells can be cryopreserved by placement of the cells in a controlled cooling enclosure (e.g., Nalgene-Nunc Mr. Frosty) pre-equilibrated at 4 °C that is placed at -80 °C or -78.5 °C for 3-4 hrs., which is then followed by storage of the cells in the vapor-phase of LN₂.
5. Following cryopreservation in CRYO-free™, for recovery, the cells should be rapidly warmed with gentle shaking in a 37 °C water bath, just until all visible ice melts (2-3 minutes maximum). Do not allow cells to incubate for prolonged periods of time at 37 °C or room temperature. **Immediately dilute cells (2-fold) in cell culture medium** and temporarily store cells at 2-8 °C (e.g., on ice) before placing into subculture. If required, rapidly count viable and non-viable cells (e.g., trypan blue exclusion) while the cells are on ice and subculture cells at the desired density using your individual protocol. When placed into subculture, CRYO-free™ cell suspensions should be diluted at least a 75-fold.

AVANTBIO LIMITED USE AGREEMENT

By acceptance of AvantBio's products (e.g. CRYO-free™ 2.5 or 5% DMSO, HKSdaFREE™, HKGE™, HFSdaFREE™, HFGE™, HFGE2™) and any other similar or dissimilar AvantBio products, whether purchased or received free of charge (herein "the transferred products"), the buyer or transferee (herein "the recipient") agrees to the terms of this Limited Use Agreement for the products, as follows:

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