CryoProtect**Pure**®

# Usage and Cryopreservation Operating Procedure

1. Isolate the cells to be cryopreserved in a suspended state using mechanical means or enzymatic digestion and collect in a conical tube.
2. Centrifuge cells to create a cell pellet. Depending upon the type of cells used, centrifugal speed and time of centrifugation may have to be adjusted and can be anywhere from 160 g to 300 g and from 5 mins to 15 mins. Use of a refrigerated centrifuge is recommended if centrifugation is carried out for longer duration of time.
3. Discard the supernatant - *Note: Remove as much culture media as possible, to reduce dilution of the CryoProtect**Pure**. The conical tubes can be inverted on a sterile surface to effectively discard the supernatant.*
4. Add 2 mL of cold (2-8°C) CPA solution XXX to the cell pallet and gently disrupt the cell pallet using pipet tip. Collect 20 uL of the cell sample for counting. Desired cell concentration for freezing may depend on the specific application. However, in general 1 – 10 million cells/mL are concentration ranges that can commonly be used. The cell samples suspended in *CryoPrtotect**Pure** s*hould then be transferred to a cryogenic tube (1-2 mL) and processed for freezing.
5. Freezing: In general, a two-step freezing process should be used. In the first phase the temperature of the cryogenic tubes are lowered at a rate of -1°C/min followed by storage at liquid nitrogen temperatures (either vapor phase of liquid phase). The lowering of temperature can be done in various different ways as described below:
    *a.* Controlled rate cooler: Use a controlled rate cooler to freeze at (-1°C/min). The actual cooling rate may be varied from 1 - 5°C/min depending upon the application need.
    *b.* Freezing devices or isopropanol container can be used to freeze the samples by placing them at -80°C freezer overnight.
6. Following initial Store samples at liquid nitrogen temperatures (below -130°C).
7. Thawing: Samples are required to thaw as quickly as possible prior to use in culture conditions to avoid re-crystallization injury. Passive thawing is not recommended.
8. Upon thawing dilute cell/ CryoProtect**Pure** mixture immediately with appropriate culture media at 37°C.
    a. A cell pallet can be created by centrifugation and the supernatant discarded, and cells resuspended in culture media.
    b. The samples can be diluted further using cell culture medium using a ratio of 1:10 or more (sample to media).

CryoProtect**PURE** is shipped at ambient temperature. Upon receipt, store at 2° - 8°C until ready for use.

Additional usage and cryopreservation support is available at info@aicryo.com or 877-768-0081.

Ad Infinitum
Cell Preservation Technologies

455 E Cady Street, Northville, MI 48167 USA

877-768-0081 | aicryo.com | info@aicryo.com