

Process Protocol for Cell Isolation from Vascularized Tissues

- I. Tissue Preparation Procedure at harvest site (at room temperature).
 - a. To prepare the cells in the organ for cold environment, perfuse or flush the tissue with room temperature (18 to 22°C) HypoThermosol®-Purge to remove native fluids from the tissue (i.e. blood)
 - b. Re-perfuse the tissue with cold (2-8°C) HypoThermosol®-FRS to replace the HTS-Purge in the tissue for cold storage
 - c. Bathe the tissue in cold (2-8°C) HTS-FRS for storage and transportation.
 - d. Transportation and storage of the organ should be done under hypothermic conditions (2-8°C).

- II. Cell Harvest (Standard Practice)
 - a. Remove tissue from storage; perfuse the tissue with HypoThermosol® base digestion medium to remove the preservation solution
 - b. Perfuse tissue with digestion cocktail solution
 - c. Dissociate the tissue into isolated cells
 - d. Screen sample to purify cell isolates
 - e. Centrifuge samples to collect viable cells
 - f. Re-suspend as necessary in culture media (for cell culture) or in CryoStor™ (for cryopreservation) or HTS-FRS for suspended cell short term storage (hypothermic storage)

- III. Cryopreservation Protocol
 - a. Place specimen into CryoStor™
 - b. Suspend cell pellet directly in cold (2-8°C) CryoStor™
 - c. Transfer sample to cryovial
 - d. Incubate samples at 2-8°C for 10 min
 - e. Freeze samples following standard protocol (1°C/min)
 - f. Controlled rate freezer
 - i. Nalgene “Mr. Frosty” Isopropanol bath freezing container
 - ii. 2-step mechanical freezer protocol (2 hours at -20°C / 2 hours at -80°C)
 - iii. Transfer samples to liquid nitrogen for storage

- iv. If cells are being plated for culture and subsequent utilization, plates can be placed into hypothermic storage (2-8°C) after a day or two of culture for short-term storage. This allows for an expanded window of utilization. Simply replace cell culture media with HypoThermosol®- FRS and place plated cells into the cold for 1-3 days. Following storage, remove cells from cold, replace the HypoThermosol®- FRS with culture media and place cells in incubation. After a recovery interval, the cells will be ready for utilization in any number of applications.

IV. Thaw

- a. Standard practice
- b. Remove sample from liquid nitrogen and immediately place into 37°C H₂O bath for 2-4 min to warm samples until just thaw (cryovial should still feel cold)
- c. Gently agitate sample during the thawing interval to achieve uniform thawing of the sample
- d. Once ice has melted immediately transfer samples to a sterile environment and dilute in 37°C culture media (1:12 dilution ratio) for cell culture

V. Testing

- a. Apply standard assays
- b. Assessment immediately post-thaw tends to render incomplete and inaccurate data regarding sample viability and function; therefore, it is recommended that viability assessment is performed 24 to 48 hours post-thaw.
 - i. Note: viability and yield assessment immediately following thawing may be helpful in evaluating the extent of delayed onset cell death (ie. when comparing 1-hour post-thaw values to 24-hours post-thaw values)
 - ii. When determining preservation efficacy, make sure assessment is performed with careful attention and comparison of both yields and viability between pre-freeze values, post-thaw values, and 24-48 hrs post-thaw. This will allow for an accurate determination of sample status and preservation efficacy.

For questions regarding this protocol or immediate assistance, please call BioLife Solutions Research and Technical Personnel (866)-4BIOLIFE (866-246-5433)