Rev. 2

ScienCell™
Research Laboratories

StemCryo™
(StemCryo; 50 mL)
Catalog #0153

Product Description
StemCryo™ is a chemically defined, serum-free, and animal component-free cryopreservation medium formulated for the cryopreservation of human pluripotent stem cells, other stem cells and delicate cell types. Engineered to support high cell viability and recovery, StemCryo ensures optimal protection of cells and tissues during freezing, thawing, and long-term storage at ultra-low temperatures (-80°C to -196°C).

- Ready-to-use formulation
- Free of serum and animal-derived components
- Contains 10% dimethyl sulfoxide (DMSO)
- Subjected to rigorous quality control, including sterility, endotoxin, and cell-based performance testing

Product Use
StemCryo is for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage
Store the StemCryo at -20°C. Once thawed, the product may be stored at 4°C for up to one month.

Shipping
Dry ice.

Procedure
Cryopreservation Protocol

1. Sanitize the exterior of the StemCryo container using 70% ethanol or isopropanol prior to opening.

2. Prepare a single-cell suspension following a protocol appropriate for your cell type. Centrifuge to collect the cells as a pellet. Gently aspirate the supernatant using a pipette, leaving a minimal volume to avoid disturbing the cell pellet. Gently resuspend the pellet by tapping the tube.

   Note: *When cryopreserving human pluripotent stem cells, maintaining the cells as large clumps rather than dissociating them into single cells is generally preferred to enhance post-thaw viability. Allow the clumps to settle by gravity at the bottom of the tube, then carefully aspirate the supernatant using a pipette, leaving a minimal volume to avoid disturbing the cell aggregates.*

3. Add pre-chilled (2–8°C) StemCryo medium to the cell pellet. Mix gently but thoroughly.
4. Transfer the cell suspension into cryovials.
5. Cryopreserve the cells using a controlled-rate cooling method (~ -1°C/min) in an isopropanol-based freezing container or a Styrofoam box at -80°C overnight. Transfer to long-term storage in liquid nitrogen.

   Note: *Long-term storage at -80°C is not recommended.*

Thawing Protocol

1. Pre-warm your preferred culture medium in a 37°C water bath.
2. Decontaminate the cryovial exterior with 70% ethanol or isopropanol.
3. In a biosafety cabinet, slightly loosen the cryovial cap to release internal pressure, then resecure.
4. Rapidly thaw the vial in a 37°C water bath with gentle agitation. Do not fully submerge the vial. Remove when only a small ice crystal remains.
   Do not vortex.
5. Wipe the vial exterior again with 70% ethanol or isopropanol.
6. Immediately dilute the thawed cell suspension at a minimum 1:20 ratio with pre-warmed culture medium and plate the cells at the desired density.

   Note: *At this dilution, residual DMSO concentration is tolerable for most cell types to survive overnight. In many cases, direct seeding without centrifugation is preferred, as the mechanical stress from centrifugation may induce greater stress than diluted DMSO exposure. If DMSO removal is essential for your application, centrifuge the cell suspension at 200 × g for 5 minutes at room temperature (15–25°C). Carefully aspirate the supernatant, leaving a small amount of medium to preserve the pellet. Resuspend cells by gently tapping the tube. Add the appropriate volume of fresh medium, mix gently, and plate the cells at the desired seeding density.*
7. Allow the cells to attach overnight, then perform a complete medium change with fresh culture medium the following day.

Caution: If handled improperly, some components of this product may present a health hazard. Take appropriate precautions when handling this product, including the wearing of protective clothing and eyewear. Dispose of properly.