

Obatala Sciences' Protocol 103 How Do I Cryopreserve Culture-Expanded Cells from Obatala Sciences?

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Reagents, Materials, and Equipment

- Purchase Obatala Sciences' Human Adipose-Derived Stromal/Stem Cells (Catalog #OS-101) or equivalent cryopreserved primary cell product
- Obatala Sciences' Cryopreservation Medium (Catalog #OS-008) or medium of choice
- Obatala Sciences' StromaQual™ Stromal Medium (Catalog #OS-001) or medium of choice
- ♦ Phosphate buffered saline (1X) or equivalent product
- ♦ 1.5 ml or 2 mL cryovials
- Controlled cooling rate freezing container suitable for cryovials
- ♦ BSL2 Biological Safety Cabinet
- Benchtop centrifuge
- ♦ -80°C freezer
- Liquid nitrogen dewar or equivalent long term storage equipment

General Requirements

- 1. All personnel should be trained and certified by the Principal Investigator regarding Universal Precautions and Handling of Bloodborne Pathogens.
- All procedures should be conducted by investigators using appropriate personal
 protective equipment at all times. Any waste materials should be decontaminated
 (bleached) and disposed of using appropriate biohazard waste containers.
- 3. Wear protective eyewear during handling of cryovial(s).

Protocol

Initial Handling of Obatala Sciences' Products

- 1. Purchase and receive Obatala Sciences' Human Adipose-Derived Stromal/Stem Cells (Catalog #OS-101) or equivalent cryopreserved primary cell product.
- 2. When you receive the package containing your Obatala Sciences' cellular product(s), remove the cryovial(s) of cells from the dry ice using appropriate safety procedures.
- 3. For immediate use, thaw and seed the cryovial of cells as described in Obatala Sciences' Protocol 101.
 - a. For intermediate storage, transfer the cryovial(s) into an appropriate freezing container for controlled cooling and place in a -80°C freezer.
 - b. For long term storage, transfer the cryovial(s) into a liquid nitrogen dewar.
- 4. Harvest cells as described in Obatala Sciences' Protocol 102.

Additional Recommendations for Handling and Use of Obatala Sciences' Products

- 1. Obatala Sciences does not recommend passaging primary cell products beyond passage 3 (P3).
- 2. Obatala Sciences does not recommend exceeding 80% confluency between passages.

Cryopreserving Culture-Expanded Cells from Obatala Sciences

- 1. After harvesting adherent cells, retrieve a cell pellet and resuspend pellet in Obatala Sciences' StromaQual™ Stromal Medium (Catalog #OS-001) or medium of choice in as described in Obatala Sciences' Protocol 102.
- According to your laboratory's standard operating procedures, determine the relative percentage of live cells and dead cells to determine total live cells and viability (%).
 - a. A hemocytometer or automatic cell counter may be used.
 - b. For hSVF cells, we recommend an acridine orange/propidium iodide (AO/PI) viability stain.
 - c. For hASCs, we recommend trypan blue viability stain.
- 3. Centrifuge the total volume of cells at 300 x g (1200 rpm) for 5 minutes at room temperature.
- 4. Return the centrifuge tube to the BSL2 biological safety cabinet and aspirate the supernatant from the pellet.
- Utilizing the total number of live cells as determined above, resuspend the cell pellet in Obatala Sciences' Cryopreservation Medium (Catalog #OS-008) at 10⁶ cells per mL.
 - (Note: Move quickly once cells are suspended in Obatala Sciences' Cryopreservation Medium (Catalog #OS-008) to minimize the exposure of the cells to the media at room temperature).
- 6. Aliquot 1 mL containing 10⁶ cells in Obatala Sciences' Cryopreservation Medium to individual 1.5 mL or 2 mL cryovials. Perform this step expeditiously.

- 7. Seal cryovial lids tightly.
 - (Note: This step is critical to avoid the leakage of liquid nitrogen into the cryovials. If the seal on the cryovial is not properly maintained, there is an opportunity for contaminants to spread between vials. Furthermore, if liquid nitrogen is present inside the vials when the cryovials are thawed, it can create an explosive force when the gas expands inside the vial!).
- 8. Transfer cryovials into a control cooling rate freezing container suitable for cryovials and place in a -80°C freezer.
- 9. After 24 hours, transfer cryovials from freezing container to a liquid nitrogen dewar for long term storage.

Remember, any laboratory that mentions Obatala Sciences' products by name in a publication is eligible for a 10% discount on their next order! We appreciate not only your business but your endorsement of our products!

Appendix A: Cryopreservation of Obatala Sciences' Cells Workflow

Step 1-Initial Handling

Step 2-Thawing and Seeding

Step 3-Maintenance and Harvest

Step 4-Cryopreservation

Upon receipt, transfer the cryovial(s) to the appropriate storage conditions.



For intermediate storage, place in a temperature-controlled container at -80°C. For long term storage, transfer to liquid nitrogen.

Remove the cryovial(s) from the storage location and transfer to a 37°C water bath for 1 minute.



After 1 minute, observe for signs of thawing. Immediately transfer the cryovial(s) to a BSL2 BSC once ice crystals are no longer visible.



Transfer the contents to a 50 mL conical tube.



Immediately add 4 mL of StromaQualⁿ dropwise to the conical tube.



Seal the cap. Centrifuge at 300xg (1200 RPM) for 5 minutes to retrieve a cell pellet.



Aspirate the supernatant.



Add 1 mL StromaQual™ to the conical tube and resuspend by pipetting.



Perform a cell count



Seed the cells at a density of 10² to 3x 10⁴ per square centimeter with StromaQual™.



Transfer the flask humidified 5% CO₂,

Proceed with feedings 3x per week with prewarmed media until cells reach optimal confluency.



On day of harvest, transfer the flask(s) or plate(s) to a BSL2 BSC.



Aspirate the media . Follow by 2 x washes with warm, sterile 1X PBS.



Add pre-warmed 0.05% trypsin/EDTA to the flask(s) or plate(s) . Tilt the vessel(s) to ensure the entire growth area is covered.



Transfer to a humidified 5% CO₂, 37°C for 5 minutes.



Once only ~10% of the cells remain attached, transfer the vessel(s) back to a BSL2 BSC. If >50% are still attached, proceed with additional incubation. Do not exceed 8 minutes.



Add an equivalent volume of StromaQual™ to the vessel(s) to neutralize the trypsin. Swirl the vessel(s) to ensure neutralization.



Pipet the solution several times to collect all non-adherent cells.



Transfer to a conical tube and centrifuge at 300xg (1200 RPM) for 5 minutes.



Transfer to a BSL2 BSC and aspirate all supernatant from the cell pellet. Resuspend in 1 mL StromaOual™.



Perform a cell count.



Further dilute cell suspension in StromaQual™ to obtain desired cell concentration or centrifuge at 300xg (1200 RPM) for 5 minutes if proceeding with cryopreservation. After cell count and retrieval of cell pellet, aspirate the supernatant and resuspend the cell pellet in Cryopreservation Media at 106 cells per mL.



Quickly transfer 1 mL containing 10⁶ cells to individual 1.5 or 2 mL cryovial tubes.



Seal the cryovials tightly.



Transfer the cryovials to a controlled cooling rate freezing container and place in a -80°C.



After 24 hours, transfer cryovials to a liquid nitrogen tank for long term storage.

Appendix B: Troubleshooting

Problem	Reason	Solution
Cells are adherent after 5 minutes incubation with 0.05% Trypsin/EDTA	Cells over overconfluent	Extend the incubation period to 7 minutes (do not exceed 8-10 minutes)
	Cells were not washed with 1X PBS prior to trypsinization	Wash the flask with warm, sterile 1X PBS prior to trypsinization
	Trypsin has undergone multiple heating cycles	Use a new lot of trypsin and/or store smaller aliquots to avoid multiple heating cycles