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.
2. 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).

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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.

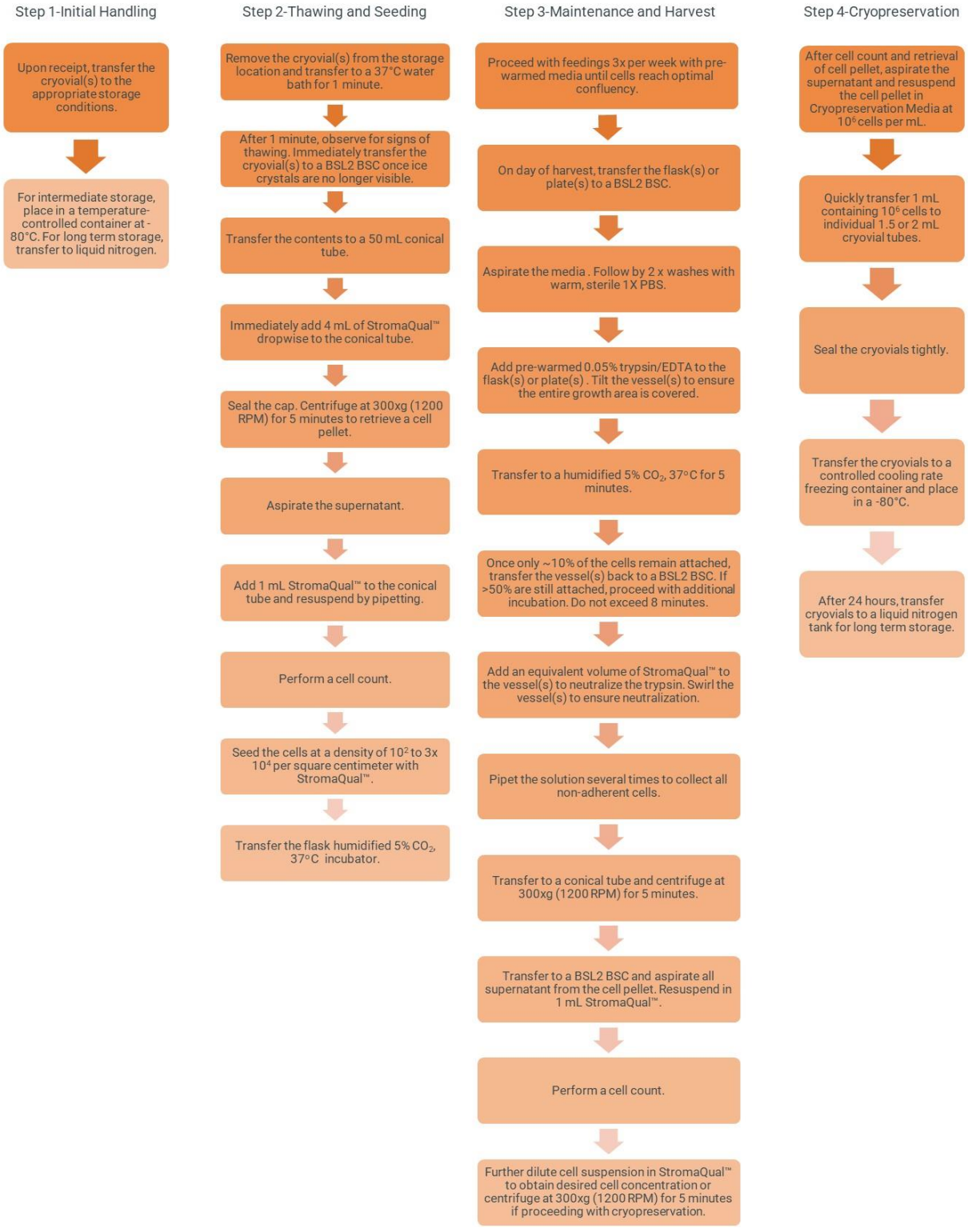
Cryopreserving Culture-Expanded Cells from Obatala Sciences

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.
2. 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.
5. Utilizing the total number of live cells as determined above, resuspend the cell pellet in Obatala Sciences' Cryopreservation Medium (Catalog #OS-008) at 10^6 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 10^6 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



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