Obatala Sciences' Protocol 101
How Do I Thaw Cryovials of Cells from Obatala Sciences?

Written by: Obatala Sciences' Scientific Team
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Reagents, Materials, and Equipment

- Obatala Sciences' Human Adipose-Derived Stromal/Stem Cells (Catalog #OS-101) or equivalent cryopreserved primary cell product
- Obatala Sciences' StromaQual™ Stromal Medium (Catalog #OS-001) or medium of choice
- BSL2 Biological Safety Cabinet
- Water bath or equivalent equipment
- 37°C, 5% CO_2 incubator
- Benchtop centrifuge
- 70% ethanol
- Sterile paper towel or kimwipe
- Conical centrifuge tube
- Flask, multi-well plate, or equivalent plasticware suitable for cell culture
- Micropipette and pipette tips
- Serological pipettes
- Pipette controller

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).

Obatala Sciences, Inc.
2000 Lakeshore Dr. #4020
New Orleans, LA 70148
504-300-0266
www.obatalasciences.com

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, proceed with thawing the cryovial(s) as detailed below.
    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.

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.

Thawing Cryopreserved Vials of Obatala Sciences' Primary Cell Products

1. Place individual vials in 37°C water bath for 1 minute to agitate the cryopreservation medium and initiate the thawing process.
   (Note: For safe handling, do not process more than 2 vials at any one time!)
2. After 1 minute, observe the cryovials for signs of thawing. Continue agitation until ice crystals disappear from solution and cryovial is just thawed.
3. Once thawed, rinse the external surface of the vial with 70% ethanol and dry with a sterile paper towel or kimwipe. Transfer the cryovial to a BSL2 biological safety cabinet.
4. Inside the biological safety cabinet, carefully open the cryovial. Transfer the contents of the vial to a conical centrifuge tube using a micropipette.
5. Adding dropwise, dilute the contents of the vial slowly by adding of 4 mL of Obatala Sciences' StromaQual™ Stromal Medium (Catalog #OS-001) or growth medium of choice. Pipette cell suspension multiple times to disperse cells and break up any tissue fragments.
   (Note: Why do I need to add the Obatala StromaQual™ Stromal Medium dropwise? We cryopreserve the cells in the presence of a cryoprotective agent. If we dilute the concentration of the cryoprotective agent too fast, the cells cannot equilibrate the small molecule across their membranes. When that happens, the recovered cells are more likely to display a low viability. So, to keep your cells happy, do not dilute them too fast!)
6. Seal the cap on the centrifuge tube. Transfer the centrifuge tube to a bench top centrifuge or equivalent and centrifuge at 300 x g (1200 rpm) for 5 minutes at room temperature.
7. Return the centrifuged tube to the biological safety cabinet and observe that a distinct and intact pellet has been retrieved. Carefully aspirate the supernatant from the cell pellet.

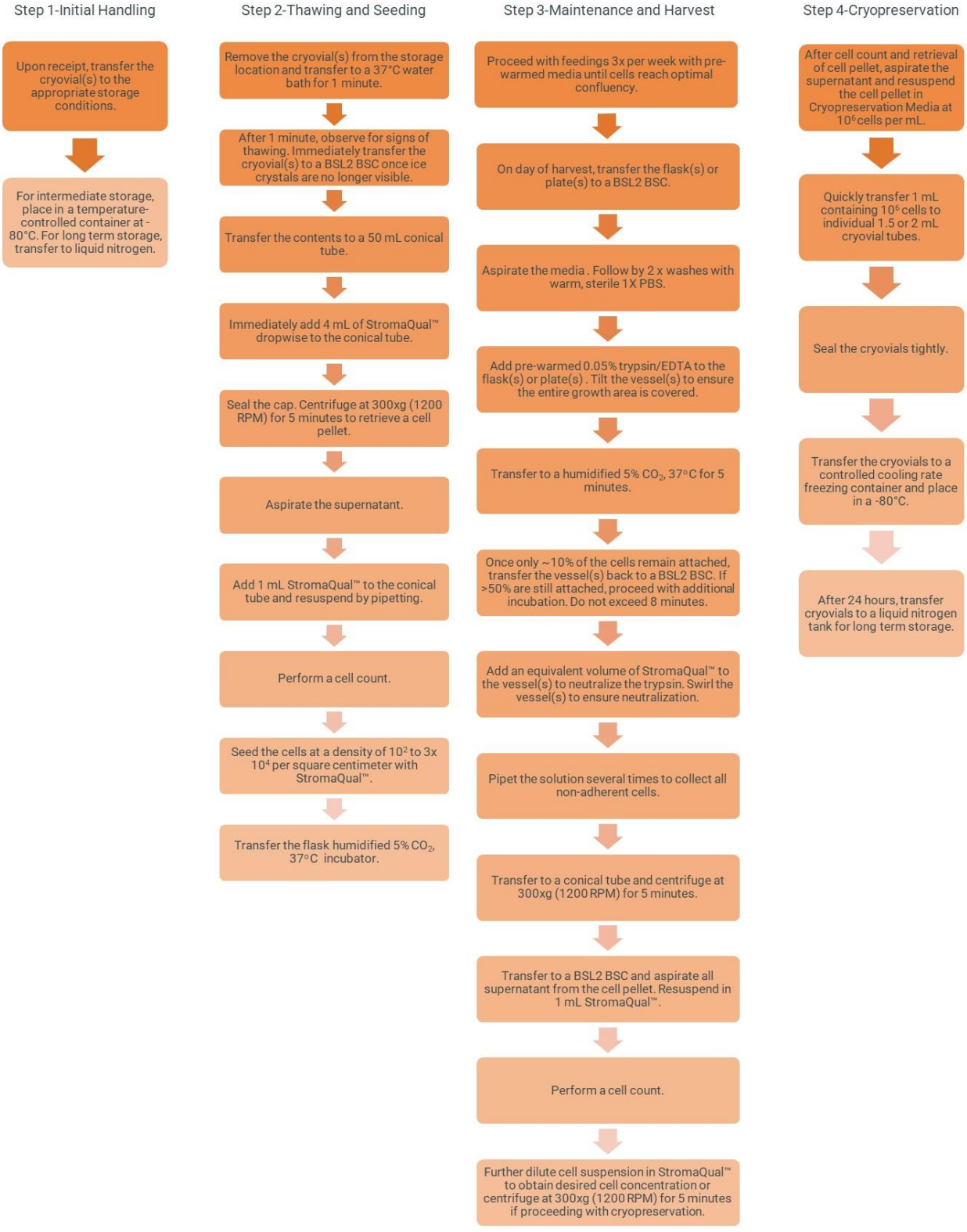
8. Resuspend pellet of cells in a volume of 1 mL of Obatala Sciences' StromaQual™ Stromal Medium (Catalog #OS-001) or medium of choice.
9. According to your laboratory's standard operating procedures, determine the relative percentage of live cells and dead cells to determine total live cells and viability (%).
    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.
10. Seed the primary cells at a recommended density of 10^2 to 3 x 10^4 per square centimeter with Obatala Sciences' StromaQual™ Stromal Medium (Catalog #OS-001) or growth medium of choice. Optimal seeding density should be empirically determined for each cell type and growth area.
    a. We recommend maintaining the cells with feedings every second day or three times per week (Monday, Wednesday, Friday) until they reach the desired degree of confluency.

Recommended Protocols

Obatala Sciences' Protocol 102-How Do I Harvest Adherent Cells from Obatala Sciences?
Obatala Sciences' Protocol 103-How Do I Cryopreserve Culture-Expanded Cells from Obatala Sciences?

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: Handling, Thawing, and Seeding of Obatala Sciences' Cells Workflow



Appendix B: Troubleshooting

Problem	Reason	Solution
Low count and/or poor viability observed with cell count	Extended thawing period	Continuously observe the cryovial when thawing. Do not leave unattended in a water bath.
	Media not added dropwise	Add media dropwise to the cell solution for adequate equilibration.
	Cell suspension not homogenized	Pipet up and down several times to ensure the cell suspension is homogenized prior to removing a volume for counting.