Obatala Sciences' Protocol 203
How Do I Induce Osteogenesis in 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)
- Obatala Sciences' OsteoQual™ Osteogenic Differentiation Medium (Catalog #OS-003)
- 70% ethanol
- Sterile paper towel or kimwipe
- Conical centrifuge tube
- Multi-well plate, or equivalent plasticware suitable for cell culture
- BSL2 Biological Safety Cabinet
- Water bath or equivalent equipment
- 37°C, 5% CO_2 incubator
- Benchtop centrifuge
- 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.

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.

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New Orleans, LA 70148
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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.

Inducing Osteogenesis in Cells from Obatala Sciences

5. 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.
6. 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.
7. Centrifuge the total volume of cells at 300x g (1200 rpm) at room temperature for 5 minutes.
8. Return the centrifuge tube to the BSL2 biological safety cabinet and aspirate the supernatant from the pellet.
9. Determine the desired density of cells and resuspend pellet in Obatala Sciences' StromaQual™ Stromal Medium (Catalog #OS-001) at desired concentration.
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.
    (Note: Density at the time of plating will determine the length of time in culture before cells reach confluence).
11. Monitor cells in culture expansion until they reach optimal confluency. Maintain cultures with feedings every 2-3 days with Obatala Sciences' StromaQual™ Stromal Medium (Catalog #OS-001) or growth medium of choice.
12. When optimum degree of confluency is reached, remove 75-90% of the StromaQual™ (Catalog #OS-001) volume and replace with an equivalent volume of Obatala Sciences' OsteoQual™ Osteogenic Differentiation Medium (Catalog #OS-003).
13. Maintain cells in Obatala Sciences' OsteoQual™ for up to 28 days, feeding every 2-3 days.

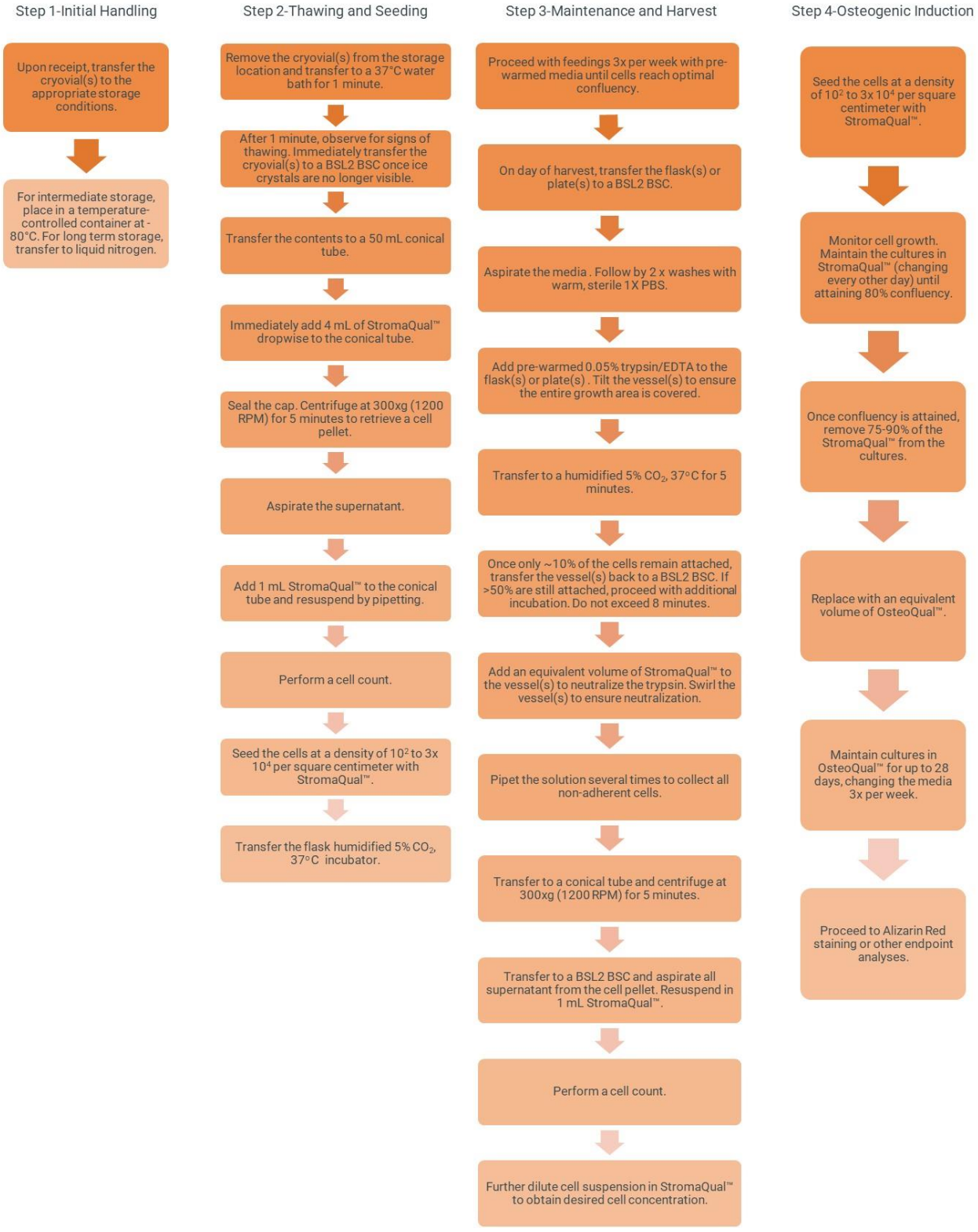
14. Monitor cells microscopically for appearance of areas of mineralization, which appear under phase contrast microscopy as densely packed areas of cell growth resembling small mounds surrounded by a monolayer of cells.
    (Note: Osteogenic differentiation has occurred when areas of mineralization appear under phase contrast microscopy).
    a. For staining of osteogenic-differentiated cells, refer to Obatala Sciences' Protocol 204.

Recommended Protocols

Obatala Sciences' Protocol 101–How Do I Thaw Cryovials of Cells from Obatala Sciences?
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?
Obatala Sciences' Protocol 204–How Do I Stain Osteogenic-Differentiated Cells with Alizarin Red?

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: Osteogenic Induction of Cells from Obatala Sciences Workflow



Appendix B: Troubleshooting

Problem	Reason	Solution
No signs of osteogenesis are observed	Media is expired	Use within 8 weeks of manufacture date
	Culture duration needs to be extended	Continue to culture up to 28 days in OsteoQual™