Obatala Sciences' Protocol 201
How Do I Induce Adipogenesis in Cells from Obatala Sciences?

Written by: Obatala Sciences' Scientific Team
Last Updated: January 2024

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' AdipoQual™ Differentiation Medium (Catalog #OS-002)
- 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.

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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.

Inducing Adipogenesis in 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 300x g (1200 rpm) at room temperature for 5 minutes.
4. Return the centrifuge tube to the BSL2 biological safety cabinet and aspirate the supernatant from the pellet.
5. Determine the desired density of cells and re-suspend pellet in Obatala Sciences' StromaQual™ Stromal Medium (Catalog #OS-001) at desired concentration.
6. 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).
7. 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.

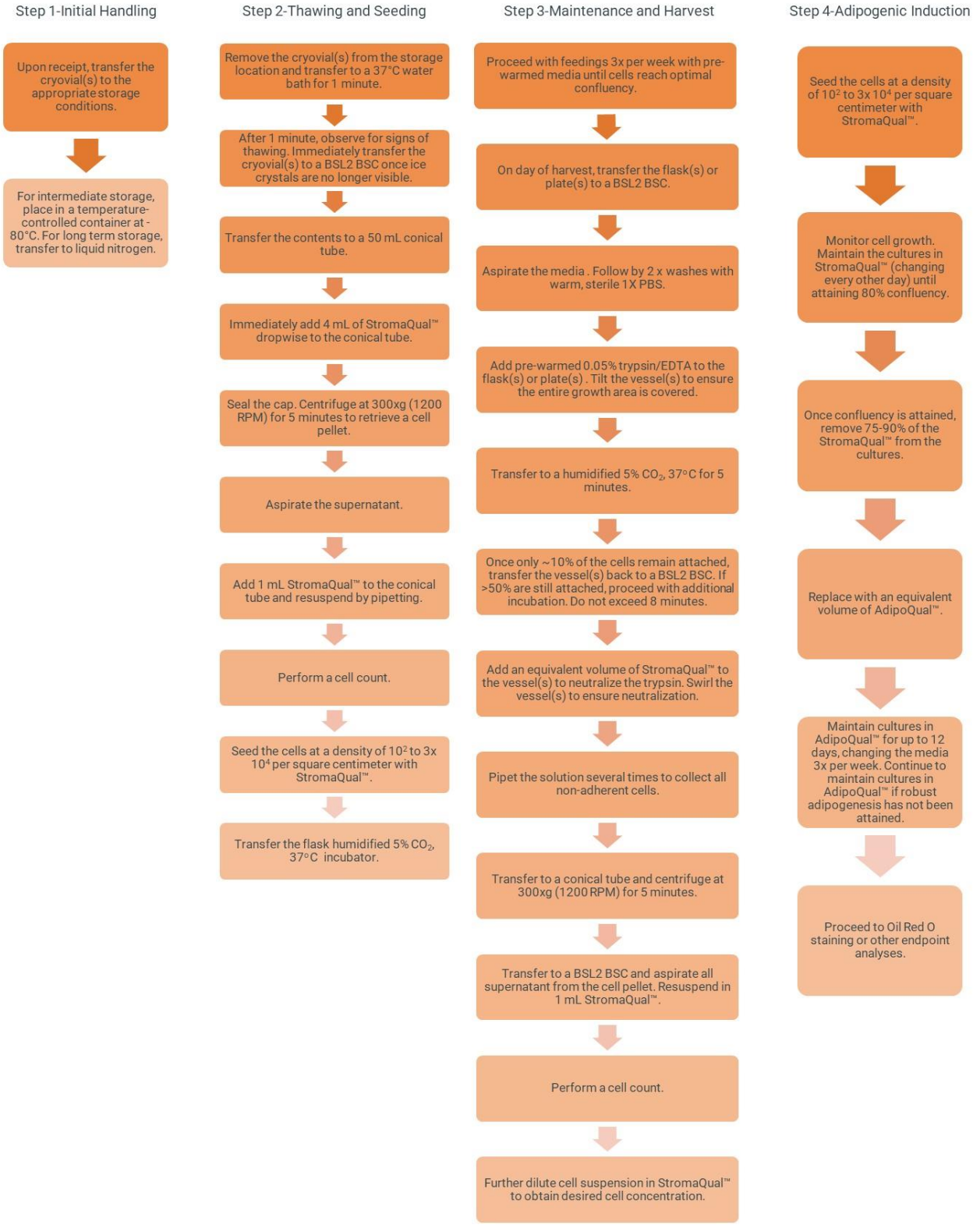
8. When optimum degree of confluency is reached, remove 75-90% of the StromaQual™ (Catalog #OS-001) volume and replace with an equivalent volume of Obatala Sciences AdipoQual™ Differentiation Medium (Catalog #OS-002).
   (Note: Do not allow the confluent cell layer to interface directly with air as that may compromise their viability and adherence after feeding).
9. Maintain cells in AdipoQual™ for up to 12 days, feeding 2-3 days.
   (Note: Cells can be maintained for a longer period of time in AdipoQual™ to achieve more robust adipogenesis. If maintained for a longer period of time, please be cognizant of the condition of the adipocytes. If lipid droplet surface area becomes too large, the cells have the tendency to lose adherence and float into the culture media).
10. Monitor cells microscopically for the appearance of lipid vacuoles, which appear under phase contrast microscopy as round, yellow globules within the cytoplasm, often surrounding the nucleus.
    (Note: Adipogenic differentiation has occurred when lipid vacuoles appear under phase contrast microscopy).
    a. For staining of adipocyte-differentiated cells, refer to Obatala Sciences' Protocol 202.

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 202–How Do I Stain Adipogenic-Differentiated Cells with Oil Red O?

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: Adipogenic Induction of Cells from Obatala Sciences 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 twice 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
Few lipid droplets are observed	Optimal confluency not attained	Ensure cultures are at least 80% confluent prior to switching to AdipoQual™
	The induction period needs to be extended	Continue to maintain cultures in AdipoQual™ past the recommended 12-day mark. Continue to change media 3x per week.