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

Written by: Obatala Sciences™ Staff
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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's 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

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 products, 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 -80C 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.

Inducing Adipogenesis in Cells from Obatala Sciences™

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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 Obatala Sciences™ Live/Dead Assay Medium (Catalog #OS-008-01) for viability stain.
    c. For hASC, we recommend trypan blue viability stain.
3. Centrifuge the total volume of cells for 5 minutes at room temperature and at 1,200 rpm (300 X g).
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 medium of choice. Optimal seeding density should be empirically determined for each cell type and growth area.
    a. 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 at least 80% 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).
    a. 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.
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.
    a. Adipogenic differentiation has occurred when lipid vacuoles appear under phase contrast microscopy.
    b. 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 Adipocyte-Differentiated Cells from Obatala Sciences™ 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!