Obatala Sciences™ Protocol 203
How Do I Induce Osteogenesis 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)
- 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

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 Osteogenesis in Cells from Obatala Sciences™

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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 Obatala Sciences™ Live/Dead Assay Medium (Catalog #OS-008-01) for viability stain.
    c. For hASC, we recommend trypan blue viability stain.
7. Centrifuge the total volume of cells for 5 minutes at room temperature and at 1,200 rpm (300 X g).
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 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.
11. 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.
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.
    a. Osteogenic differentiation has occurred when areas of mineralization appear under phase contrast microscopy.
    b. For staining of osteogenically 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 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!