Obatala Sciences™ Protocol 102
How Do I Harvest Adherent Cells from Obatala Sciences™?

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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
- Obatala Sciences' 1x Phosphate Buffered Saline (Catalog# OS-009) or equivalent product
- 0.05% trypsin/EDTA Solution
- 70% ethanol
- Sterile paper towel or kimwipe
- Conical centrifuge tube
- Flask, 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.
3. Wear protective eyewear during handling of cryovial(s).

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

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4. Proceed with harvesting protocol as outlined below after seeding the primary cells at a recommended density of 10^2 to 3 X 10^4 per square centimeter and feeding the cells with Obatala Sciences™ StromaQual™ Stromal Medium (Catalog #OS-001) or medium of choice until desired level of confluency is reached.

Harvesting Adherent Cells from Obatala Sciences™ Primary Cell Products

1. Allow the cells to reach the desired level of confluence based on microscopic examination, where the optimal level of confluency should be empirically determined.
2. Transfer the flask(s) or plasticware of choice to a BSL2 biological safety cabinet.
3. Aspirate the culture media from the flask(s) or plasticware of choice.
4. Rinse the flask(s) or plasticware of choice twice with pre-warmed (37ºC) Obatala Sciences' 1x Phosphate Buffered Saline (Catalog# OS-009) or equivalent product.
5. Add 0.05% trypsin/EDTA to the flask or plate, we recommend using 1 ml for roughly 40 to 50 square centimeters of surface area. Tilt the flask(s) or plasticware of choice to ensure that 0.05% trypsin/EDTA solution coats the entire growth area.
6. Incubate the flask(s) or plasticware of choice in a 37ºC incubator with 5% CO_2 and 85% humidity for 5 minutes. After 5 minutes, observe the cells under a microscope to verify that cells have begun lifting from the surface and are floating in solution when tapped. If fewer than ~10% of cells remain attached, proceed with the next steps.
    a. If >50% of cells appear still attached to the plate with little change in morphology or lifting when tapped, return to incubator for additional trypsinization. Observe every minute for signs of lifting until fewer than ~10% of cells remain attached.
    b. Do not allow cells to incubate in 0.05% trypsin/EDTA solution for longer than 8-10 minutes regardless of adherence. Cell viability will be impacted with prolonged exposure to 0.05% trypsin/EDTA solution.
7. Return the flask(s) or plasticware of choice to the BSL2 biological safety cabinet.
8. Add an equivalent volume of Obatala Sciences™ StromaQual™ Stromal Medium (Catalog #OS-001) or medium of choice to neutralize the volume of 0.05% trypsin/EDTA to each flask or plasticware of choice. Swirl flasks to ensure that solution is neutralized.
9. Pipet the solution inside the flask multiple times aseptically to collect all de-adherent cells from the growth area.
10. Collect volume in a conical centrifuge tube.
11. Centrifuge the cells for 5 minutes at 1,200 rpm (300 X g) at room temperature.
12. Return conical centrifuge tube to the BSL2 Biological Safety Cabinet.
13. Carefully aspirate the supernatant from the cell pellet.
14. Resuspend pellet of cells in a volume of 1 ml of Obatala Sciences™ StromaQual™ Stromal Medium (Catalog #OS-001) or medium of choice.
15. 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.

16. Further dilute the cell suspension Obatala Sciences™ StromaQual™ Stromal Medium (Catalog #OS-001) or medium of choice at the desired cell concentration for culture expansion or centrifuge the cell suspension at 1,200 rpm (300 X g) for 5 minutes at room temperature to retrieve a cell pellet in preparation for cryopreservation or other procedures.

Recommended Protocols

Obatala Sciences™ Protocol 101 – How Do I Thaw Cryovials 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!