Obatala Sciences' Protocol 202
How Do I Stain Adipogenic-Differentiated Cells with Oil Red O?

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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
- Oil Red O staining solution
- Phosphate buffered saline (1X) or equivalent product
- 4% paraformaldehyde or 10% formalin fixative
- Inverted microscope with camera
- Multi-well plate, or equivalent plasticware suitable for cell culture
- Distilled water
- BSL2 Biological Safety Cabinet
- Water bath or equivalent piece of equipment
- Micropipette and pipette tips

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.
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. Induce cells with Obatala Sciences' AdipoQual™ Differentiation Medium (Catalog #OS-002) for up to 15 days in culture as described in Obatala Sciences' Protocol 201.

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.

Staining Adipocyte-Differentiated Cells with Oil Red O

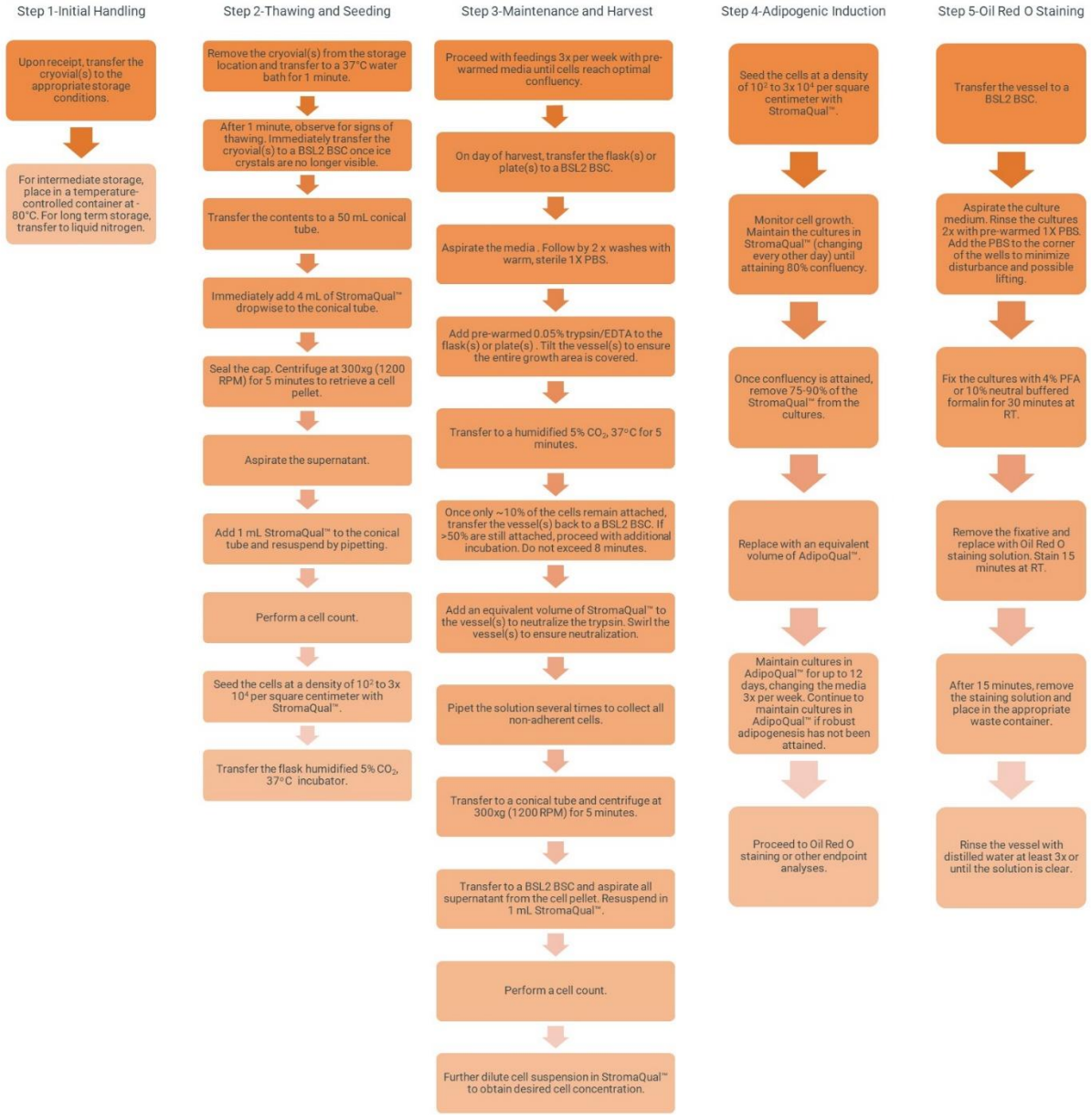
1. Transfer multi-well plate(s) of differentiated Obatala Sciences' adipocytes to a BSL2 biological safety cabinet.
2. Aspirate the media from the cells and rinse the cells twice with pre-warmed (37°C) phosphate buffered saline or equivalent product.
   (Note: Take care to pipet the solution into the corner of the wells or flask to ensure the adherent layer of differentiated cells remain intact).
3. Fix the cells in 4% paraformaldehyde or 10% formalin in phosphate buffered saline or equivalent product for a period of 30 minutes at room temperature.
4. Remove the fixative solution and replace with an Oil Red O staining solution. Add sufficient staining solution to cover the growth area of the culture.
5. Stain for 15 minutes at room temperature.
6. After a 15-minute staining period, remove the staining solution and place in an appropriately labeled chemical waste container.
7. Rinse the plate or flask with distilled water three times or until wash solution remains clear.
   (Note: Take care to pipet the solution into the corner of the wells or flask to ensure the adherent layer of differentiated cells remain intact).
8. Monitor the degree of staining through microscopic examination. All lipid vacuoles should display a red color under phase contrast microscopy. If this is not the case, re-stain the adherent cells with an Oil Red O staining solution following the same procedure outlined above.
   (Note: Run empty wells (no cells) as background control for artefactual staining of plastic well itself; these wells should be subjected to all rinsing and washing steps until completion of procedure).
9. Once sufficient staining has occurred, the degree of Oil Red O staining can be monitored photographically under phase contrast microscopy to qualitatively assess the degree of positive staining.
10. For quantitative assessment, the degree of Oil Red O staining can be monitored by capturing the entire surface area of the cultures using an imaging device (like a phone camera). The images can then be analyzed using a software program (such as NIH's FIJI/Image J) to quantitatively determine the percentage of the surface area staining positive for Oil Red O.

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 201–How Do I Induce Adipogenesis in 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!

Appendix A: Oil Red O Staining of Adipogenic-Differentiated Cells from Obatala Sciences Workflow



Appendix B: Troubleshooting

Problem	Reason	Solution
No adipocytes are observed after staining	Cells lifted during fixation	Gently add fixative to a corner of the well or plate. Do not directly add to the center.
	Cells lifted during washing	Gently add 1X PBS to a corner of the well or plate. Do not add directly to the center.