



Obatala Sciences' Protocol 204

How Do I Stain Osteogenic-Differentiated Cells with Alizarin Red?

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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
- ◆ Alizarin Red staining solution
- ◆ 150 mM NaCl
- ◆ 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% CO₂ incubator
- ◆ Inverted microscope with camera
- ◆ Micropipette and pipette tips
- ◆ Distilled water

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. Differentiate osteogenic cells for up to 28 days in culture as described in Obatala Sciences' Protocol 203.

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 Osteogenically-Differentiated Cells with Alizarin Red

1. Transfer multi-well plate(s) of differentiated cells to a BSL2 biological safety cabinet.
2. Aspirate the media from the cells. Rinse the cells with warm 150 mM NaCl for a total of three washes.
(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 cold 70% ethanol kept on ice. Fix plate for 1 hour at 4°C.
4. Remove ethanol from wells and rinse with distilled water for a total of 3 washes.
5. Cover the base of the well with Alizarin Red staining solution.
6. Stain for 10 minutes at room temperature.
7. After a 10-minute staining period, remove the staining solution and place in an appropriately labeled chemical waste container.
8. Rinse the wells five times with water 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).
9. Monitor the degree of staining through microscopic examination.
(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).
10. Photograph under phase contrast microscopy immediately for documentation at selected magnification.

Recommended Protocols

Obatala Sciences' Protocol 101–How Do I Thaw Cryovials of Cells from Obatala Sciences?

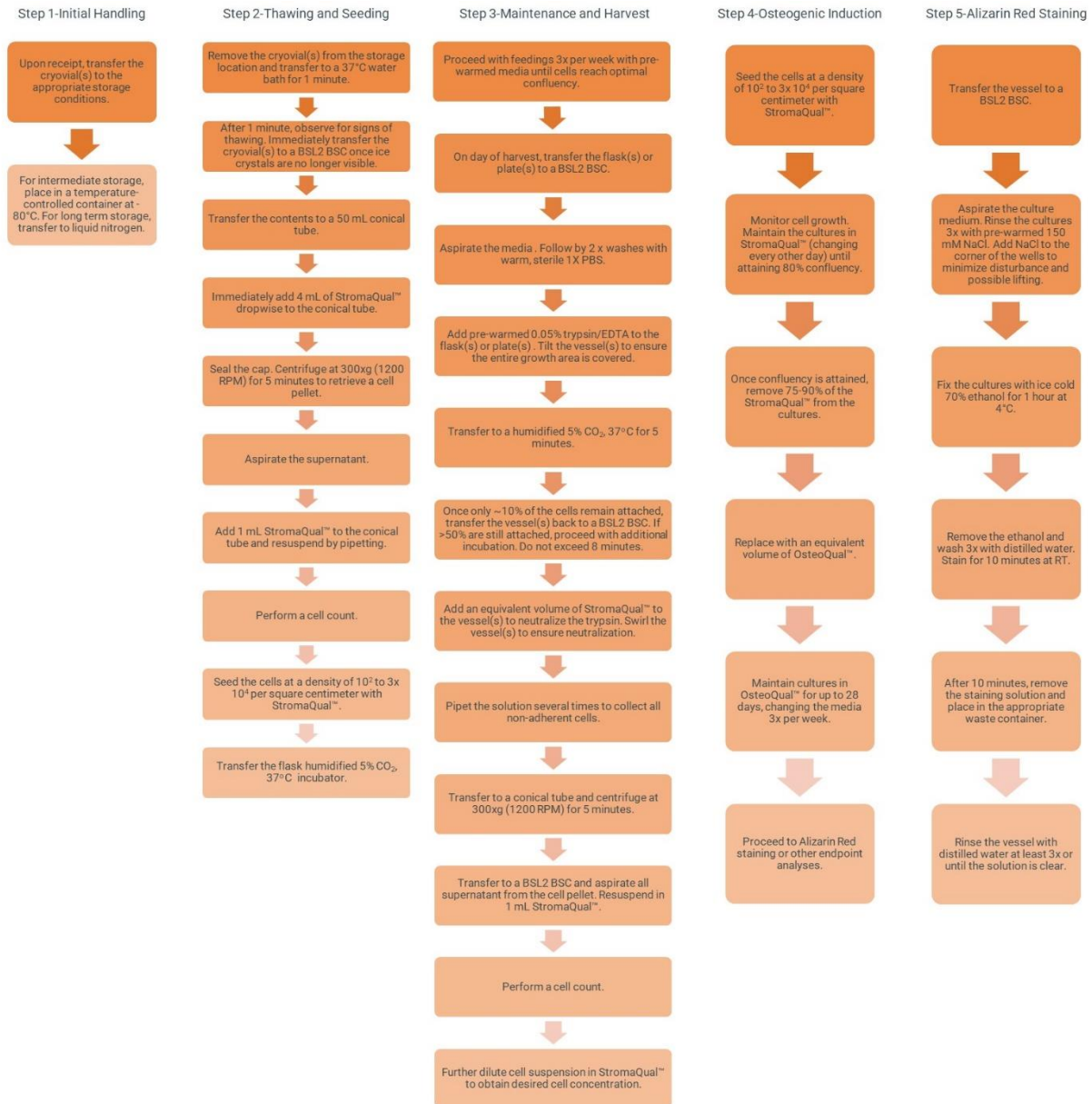
Obatala Sciences' Protocol 102–How Do I Harvest Adherent Cells from Obatala Sciences?

Obatala Sciences' Protocol 103–How Do I Cryopreserve Culture-Expanded Cells from Obatala Sciences?

Obatala Sciences' Protocol 203–How Do I Induce Osteogenesis 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: Alizarin Red Staining of Osteogenic-Differentiated Cells from Obatala Sciences Workflow



Appendix B: Troubleshooting

Problem	Reason	Solution
No osteocytes are observed after staining	Cells lifted during fixation	Gently add 70% ethanol to a corner of the well or plate. Do not directly add to the center.
	Cells lifted during washing	Gently add 150 mM NaCl to a corner of the well or plate. Do not add directly to the center.
	Induction period was too short	Extend the induction period to a full 28 days to ensure adequate calcification is attained