Obatala Sciences™ Protocol 204
How Do I Stain Osteogenic-Differentiated Cells from Obatala Sciences™?

Written by: Obatala Sciences™ Staff
Last Updated: July 2021

Reagents, Materials, and Equipment

- Obatala Sciences' Human Adipose-Derived Stromal/Stem Cells (Catalog #OS-101) or equivalent cryopreserved primary cell product
- Obatala Sciences' Alizarin Red Solution (Catalog #OS-006)
- 10% cetylpyridinium chloride monohydrate
- 150 mM NaCl
- 70% ethanol
- Sterile paper towel or kimwipe
- Conical centrifuge tube
- Multi-well plate, or equivalent plasticware suitable for cell culture
- Instrument to assess optical density

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

Obatala Sciences, Inc.
2000 Lakeshore Dr. #4020
New Orleans, LA 70148
504-300-0266
www.obatalasciences.com

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

Staining Osteogenically-Differentiated Cells from Obatala Sciences™

1. Transfer multi-well plate(s) of differentiated Obatala Sciences™ osteoblasts to a BSL2 biological safety cabinet.
2. Aspirate the media from the cells. Rinse the cells with 150 mM for a total of three washes.
    a. Take care to pipet the solution onto the side of the plate or flask so as not to disrupt the adherent layer of differentiated cells with direct contact.
3. Fix the cells in cold 70% ethanol kept on ice. Fix plate for 1 hour at 4ºC.
4. Remove ethanol from wells and rinse with water for a total of 3 washes.
5. Cover the base of the well with Obatala Sciences' Alizarin Red Solution (Catalog #OS-006).
6. Stain for 10 minutes at room temperature.
7. After 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.
    a. Take care to pipet the solutions onto the side of plate or flask to avoid disturbing the fixed adherent cell layer.
9. Monitor the degree of staining through microscopic examination.
    a. 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.
11. Alternatively, to quantify the degree of Alizarin Red Staining destain by adding 50 µl of 10% cetylpyridinium chloride monohydrate (10% CPC; 1 gram in 10 ml distilled water) to each well (96 well plate). Destain for approximately 30 minutes. Monitor destaining from cells by eye or under microscopic analysis.
12. Read OD_{540} on each plate. Subtract out background staining from blank wells to correct for artefactual staining. Determine staining under osteogenic or experimental conditions relative to control stromal cells without osteogenic induction.

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!