Obatala Sciences™ Protocol 202
How Do I Stain Adipocyte-Differentiated 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' Phosphate Buffered Saline 1x (Catalog #OS-009) or equivalent product
- Obatala Sciences' Oil Red O Staining Solution (Catalog #OS-005) or equivalent product
- 4% paraformaldehyde or 10% formalin fixative
- 96 well plate reader or equivalent instrument
- 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. Adipogenically differentiate cells for up to 15 days in culture as described in Obatala Sciences™ Protocol 201.

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

Staining Adipocyte-Differentiated Cells from Obatala Sciences™

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 (37ºC) Obatala Sciences' Phosphate Buffered Saline 1x (Catalog #OS-009) or equivalent product.
    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 4% paraformaldehyde or 10% formalin in Obatala Sciences' Phosphate Buffered Saline 1x (Catalog #OS-009) or equivalent product for a period of 30 minutes.
4. Remove the fixative solution and replace with Obatala Sciences' Oil Red O Staining Solution (Catalog #OS-005) or equivalent product. Add sufficient Staining Solution to cover the growth area of the culture.
5. Stain for 15 minutes at room temperature.
6. After 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.
    a. Take care to pipet the solutions onto the side of plate or flask to avoid disturbing the fixed adherent cell layer.
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 Obatala Sciences' Oil Red O Staining Solution (Catalog #OS-005) following the same procedure outlined above.
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.
    a. Alternatively, the degree of Oil Red O Staining can be monitored by eluting the retained Oil Red O Stain with the addition of a minimal volume of isopropanol per well. Immediately recover the isopropanol volume. Read the Optical Density 540 on a 96 well plate reader or equivalent instrument to determine the level of Oil Red O Staining. Designate a well with cells cultured in the absence of adipogenic differentiation as a baseline control and express the retained Oil Red O Staining as a level relative to this value, set as "1".

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™?

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!