LaCell's "How To" Protocol 105
How Do I Stain Adipocyte Differentiated Cells from LaCell with Oil Red O?

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Date: July 10, 2015

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. Purchase LaCell Catalog # hASC-01D or equivalent cryopreserved primary cell product.
4. Thaw and seed the cryovial of hASC-01D as described in LaCell Protocol 101.
5. Harvest cells as described in LaCell Protocol 102.
6. Differentiate adipogenic cells for up to 15 days in culture as described in LaCell Protocol 104.
7. Take plates or flasks of differentiated LaCell adipocytes and transfer to a BSL2 biological safety cabinet.
8. Aspirate the media from the cells using aseptic technique. Rinse the cells twice with pre-warmed 37^{o} C LaCell's Phosphate Buffered Saline 1 X (LaPBS1X). Take care to pipet the solution onto the side of the plate or flask so as not to damage the adherent layer of differentiated cells.
9. Fix the cells in 4% paraformaldehyde or 10% formalin in LaCell's Phosphate Buffered Saline 1 X (LaPBS1X) for a period of 30 minutes.
10. Remove the fixative and replace with LaCell's Oil Red O Staining Solution (LaORO). Add sufficient Staining Solution to cover the surface of the culture vessel. Stain for 15 minutes at room temperature.
11. Remove the Staining Solution and place in appropriately labeled chemical waste container.
12. Rinse the well or plate with distilled water three times (until clear). Take care to pipet the solutions onto the side of plate or flask to avoid disturbing the fixed adherent cell layer. Monitor the degree of staining by 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 LaCell's Oil Red O Staining Solution.
13. At this stage, the degree of Oil Red O Staining can be monitored photographically under phase contrast microscopy.
14. Alternatively, the degree of Oil Red O Staining can be monitored by scanning the entire surface area using an image device (for example, a cell phone camera) and the image analyzed using a software program (such as NIH's Image J) to determine the percentage of the surface area staining positive for Oil Red O.

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15. 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".