LaCell's "How To" Protocol 107
How Do I Stain Osteogenic Differentiated Cells from LaCell with Alizarin Red?

Written by: LaCell Staff
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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 osteogenic cells for up to 28 days in culture as described in LaCell Protocol 106.
7. Take plates 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° C 150 mM NaCl three times. **Do not use LaCell's Phosphate Buffered Saline at any time during this protocol; it will create an artifact in your data.**
9. Fix the cells in ice cold 70% ethanol. Place plate for 1 hour at 4°C.
10. Remove ethanol from the well. Rinse with water 3 times. Cover the base of the well with LaCell's Alizarin Red Solution (LaAR); use 50 µl per well on a 96 well plate. Allow to stain for 10 minutes at room temperature. Observe under a microscope to determine the extent of staining. **Run empty wells (no cells) as background control for artifactual staining of plastic well itself; these wells should be subjected to all rinsing and washing steps until completion of procedure.**
11. Rinse the wells 5 times with water. Rinse a final (6^{th}) time for 15 minutes in distilled water.
12. Photograph under phase contrast microscopy immediately at this step for documentation at selected magnification.
13. Alternatively, to quantify the degree of Alizarin Red Staining, remove water from well. 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.

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14. Read OD_{540} on each plate. Subtract out background staining in blank well to correct for artifactual staining. Determine staining under osteogenic or experimental conditions relative to control stromal cells without osteogenic induction.

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