MLPC™-UCB Clonal Cell Line
Osteogenic Differentiation of MLPC

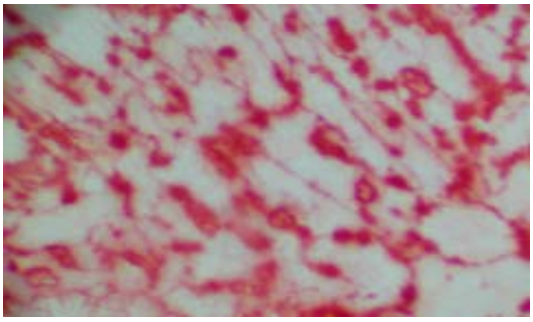
Reagents and Materials

- MSCGM, Lonza Walkersville, Inc., PT-3001
- Osteogenic Induction Medium, Lonza Walkersville, Inc. PT-3002
- LabTek II, 4-well chamber slides, Nalge Nunc, 154526
- Hanks Balanced Salt Solution, Sigma, H1641 or equivalent
- Bovine Serum Albumin, Fisher, BP1600-1 or equivalent
- Paraformaldehyde, Sigma, P-6148 or equivalent
- Alizarin Red S Stain, Sigma, A-553

Control MLPC cultures stained with Alizarin Red and observed under phase contrast microscopy.

Osteogenic Procedure

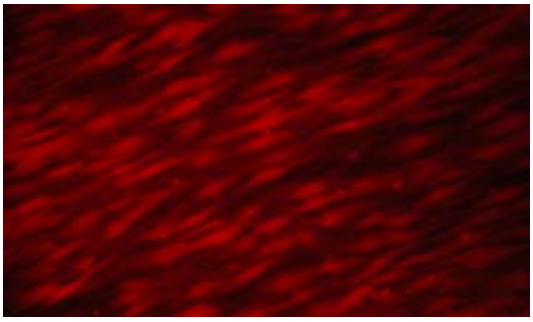
1. Plate MLPCs in non-coated 4 - well chamber slides at 1 x 10^4 cells/well in 1mL of MSCGM.
2. Incubate the cells in a 5% CO_2 atmosphere at 37°C.
3. Cells are fed every 3-4 days with MSCGM until the cultures reach confluency.
4. At confluency, continue to feed the control wells with MSCGM Medium.
5. The cells to be differentiated have their medium replaced with Osteogenic Induction Medium.
6. Feed with the above medias every 3-4 days for approximately 14 days.
7. Heavy deposition of calcium crystals in osteogenic culture was demonstrated by using a modification of the Alizarin red procedure, observing red staining of calcium mineralization by fluorescent microscopy and positive red staining of osteoblasts by phase contrast microscopy.
8. Control cultures maintain fibroblast morphology and demonstrate no mineralization.



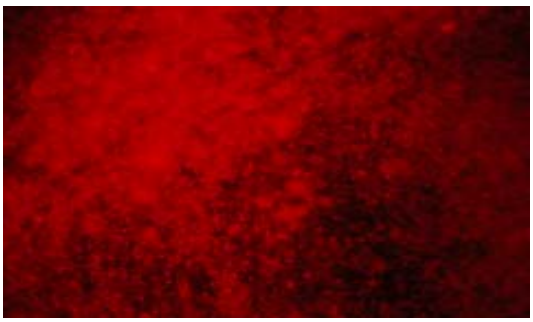
MLPC cultured in Osteogenic Induction Medium, stained with Alizarin Red and observed under phase contrast microscopy.

Alizarin Red Staining Procedure

1. Carefully remove media from all wells.
2. Wash each well 2 times with 1mL Hanks + 1% BSA.
3. Add 1mL of 4% Paraformaldehyde to each well.
4. Let the slide incubate at room temperature for 30 minutes.
5. Remove the 4% Paraformaldehyde from each well.
6. Wash each well 3 times with 1mL Hanks + 1% BSA.
7. Add 1mL of 0.1% Alizarin Red S stain to each well.
8. Let the slide incubate at room temperature for 20 minutes.
9. Remove the stain.
10. Wash each well 3 times with 1mL Hanks + 1% BSA.
11. Remove the chamber from the slide using the slide separator.



Control MLPC cultures stained with Alizarin Red and observed under fluorescent microscopy.



MLPC cultured in Osteogenic Induction Medium, stained with Alizarin Red and observed under fluorescent microscopy.