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Calcium Deposition Assay for *In Vitro* Osteogenesis

Materials

- DPBS, without calcium or magnesium – Lonza catalog # 17-516Q
- 0.5N HCl
- Calcium (CPC) Liquicolor kit - Stanbio Laboratory catalog # 0150-250
- Induced Osteogenic cultures
- Plate reader or spectrophotometer

Procedure

- Aspirate all culture medium from each well of a 6-well culture plate that contains induced or control cells to be tested.
- Rinse the cells in the plate by adding 1 ml of PBS to the side of each well, being careful to not dislodge the cells.
- Aspirate off the PBS and re-rinse, as above.
- Aspirate the second wash and add 0.5 ml of 0.5N HCl to each well.
- Scrape the cells off of the surface using a cell lifter and transfer the cells and HCl to a polypropylene tube (1.5 ml Eppendorf tube or any 2-5 ml polypropylene tube with a tight fitting cap).
- Add an additional 0.5 ml of 0.5N HCl to each well to recover any cells remaining in the well, and transfer this to the appropriate tube.
- Samples may be capped tightly and stored at -20°C for one month if they are not to be tested immediately.
- Extract the calcium from the cells by shaking the tubes on an orbital shaker for 3 - 24 hours at 4°C. If using frozen samples, allow extra time for samples to thaw.
- Centrifuge the sample tubes at 500g for 2 minutes.
- Carefully collect the supernatant with extracted calcium, without disrupting the pellet, and transfer to a new tube.
- Following the instructions provided in the Stanbio Laboratory Calcium (CPC) Liquicolor kit, prepare a standard curve with the calcium standard and determine the amount of calcium in each control and osteo-induced sample.
- Sample and assay reagent volumes may be adjusted to fit microtiter plates (200 μl) or spectrophotometer cuvettes (2 ml).
- 10 μl – 100 μl of sample is used for each calcium determination. Unused sample extract may be re-frozen for future re-assay.

References:

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