Osteocyte Differentiation Procedure

1. After incubating the prepared Adipose-Derived Mesenchymal Stem Cells for 48 hours (as described above), pre-warm the Osteocyte Differentiation Tool to 37°C in a water bath.
2. Bring a bottle of D-PBS (ATCC® 30-2200) to room temperature.
3. Remove the prepared Adipose-Derived Mesenchymal Stem Cells from the incubator and carefully aspirate the culture medium from each well.
4. Rinse the cells by gently adding 2 mL of room-temperature D-PBS (ATCC® 30-2200) to each well, then aspirating the PBS rinse from the wells while being careful not to disturb the cells.
5. Add 2 mL of the pre-warmed Osteocyte Differentiation Tool to each well. (Store the remaining Osteocyte Differentiation Tool in the dark at 2°C-8°C for later use).
6. Incubate the cells at 37°C with 5% CO2 for 3-4 days before renewing the medium.
7. When ready to renew the medium, retrieve the Osteocyte Differentiation Tool from storage and transfer the required volume to a sterile tube. (For a complete 6-well plate, this volume would be 12 mL).
8. Warm the transferred aliquot of Osteocyte Differentiation Tool to 37°C in a water bath.
9. Remove all but 1 mL of the old medium from each well containing cells. Important: DO NOT TILT the plate during aspiration or otherwise expose the monolayer to air during this or any subsequent steps.
10. Add 2 mL of fresh, pre-warmed Osteocyte Differentiation Tool to each well by pipetting the medium gently down the side of the well to keep from disturbing the monolayer or accumulated calcium crystals. (This now brings the final volume in each well to 3 mL).

Note: The monolayer of differentiating cells is under tension and extremely fragile. The cells can easily
detach from the plate and must be handled with care.

1. Repeat steps 6 through 10 every 3-4 days until the cells have been exposed to the Osteocyte Differentiation Tool for a total of 19 days.
2. Cells can be used at any phase of osteocyte differentiation as predicated upon experimental design.

Note: If curling of the edges of the monolayer is observed, the cells will detach from the tissue culture plate within 24-48 hours and should be used immediately.

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