

## Center for Musculoskeletal Research

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## Musculoskeletal Cell Core

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# Growing and Culturing OCY454 Osteocytic Cell Line (conditionally immortalized)

### Required Supplies:

- 1) Gibco FBS (US Sourced, Cat # 26140)
- 2) Gibco Anti-Anti (Life Technologies Cat #15240).
- 3) Gibco Alpha-MEM (Life Technologies Cat #12571)
- 4) Collagen I Coated Flasks (ie, BD/Corning ColCoat) (**optional**)
- 5) Freezing Media: BamBanker (Wako Cat #302-14681)
- 6) For splitting cells, sterile PBS w/o Mg and CaCl and TrypeE (Life Technologies Cat #: 12605-010)

### Protocol:

- 1) Prepare complete media: 10% Gibco FBS (U.S. Sourced), 1% Gibco Anti-Anti. To prepare, add 50 ml Gibco media and 5 ml Gibco Anti-Anti (100X) to 500 ml alpha-MEM.
- 2) If frozen stocks, follow protocol from BamBanker and plate into T-25 Collagen Coated Flasks @ 33C. Upon confluence, split and maintain cells in T-75 Collagen Coated Flasks (or regular TC flasks) @33C. Cells need to be split every ~3 days to avoid over-confluence. Cells are split when 90% confluent. If cells are let become overconfluent they express lower level of Sost/sclerostin.
- 3) Cells are routinely maintained @ 33C in collagen coated tissueware. Upon confluence, the standard PDP laboratory protocol is to plate the cells @ 100K/ml in non-collagen coated tissueware. No differentiation media or other additives are used. Three days post plating, media is changed and cells are moved to 37C. Thereafter, media is changed every 2-3 days. Routine PDP laboratory timepoint for osteocytic phenotype is 7-10 days in culture at 37C. Expected Sost expression after 7-10 days in culture at 37 C is between 28-32 Ct (with beta-actin at 16-18 Ct) for rt-PCR