Materials and Equipment

Materials

- Osteoblasts (AM005-F)
- Osteoblast Cellutions Medium (O-GRO)
- 1× PBS (Ca^{2+}-/Mg^{2+}-free),
- TrypLE Expression
- 70% isopropyl alcohol (IPA)

Equipment

- Incubator, 37°C/ 5% CO_2.
- Tissue culture vessels
- Water Bath, 37 °C
- 50.0 ml tubes
- Centrifuge
- Pipettes

Procedure

1.	Adopt aseptic practices for all steps involving cell culture.
2.	Remove cryovial from shipping container to check that it is still frozen. Transfer frozen vial to liquid nitrogen until you are ready to thaw and begin cell culture.
3.	Thaw cryovial in 37°C water bath with agitation. For details see How to Thaw Cells
4.	Transfer contents (1 to 2ml) of cryovial to 50-ml conical tube; slowly add dropwise with agitation 9ml FBS (pre-warmed at 37°C).
5.	Centrifuge 50 ml tubes 400 g at 4°C for 5 min.
6.	Remove supernatant; disperse cell pellet with O-GRO; add sufficient O-GRO to make 1 to 5ml; determine cell count. For details see How to Count Cells.
7.	Seed cells at densities 3-10 × 10^3 cells/ cm^2 in O-GRO; incubate at 37°C, 5% CO_2; replace spent medium with fresh medium every 3 to 4 days.
8.	When cells are 80% to 90% confluent, they can be subcultured.
9.	To subculture, remove medium; rinse cells with PBS (pre-warmed at 37°C); dissociate cells with TrypLE Express (37°C, 5 to 10min); quench dissociation with O-GRO; centrifuge cells 400 g at 4°C for 5 min.
10.	Remove supernatant; disperse cell pellet with O-GRO; add sufficient O-GRO to make 1 to 5ml; determine cell count.
11.	Seed cells at densities 3-10 × 10^3 cells/ cm^2 in O-GRO; incubate at 37°C, 5% CO_2; replace spent medium with fresh medium every 3 to 4 days.