**Alkaline phosphatase activity assay lysis**

**Procedure**

1. Distribute cells into 24 well plates and incubate for 50 % confluence for ALP in 2 days and 30 % confluence for ALP in 7 days.
2. Treat cells with samples in proper concentration and wait for required days.
3. Remove medium and rinse cells twice with PBS
4. Add 100 µl lysis buffer to each cell of 24 well plate
5. Transfer 50 µl of cell lysate to each well of 96 well plate
6. Add 100 µl of enzyme assay buffer and cover with aluminum foil
7. Incubate plate for 30 min for 37 °C
8. Measure the absorbance at 405 nm

**Reagent**

Cell lysis buffer

1. 0.1% Triton X-100
2. 25 mM carbonate buffer (pH 10.3)

**Enzyme assay buffer**

1. 250 mM carbonate buffer (pH 10.3)
2. 2.5 mM MgCl2
3. 15 mM p-nitro phenyl phosphate