Luminescence Assay for Alkaline Phosphatase Activity of C3H10T(1/2) Cells

Reagents

Luminometer assay plate
Lysis buffer (100 mM Tris pH 9.5, 250 mM NaCl, 25 mM $MgCl_2$, 1% Triton X-100)
CDP-Star Chemiluminescence reagent (PerkinElmer)

Procedure

1) Seed 96-well tissue culture plate with 5000 C3H10T1/2 cells/well in DMEM containing 10% fetal bovine serum and penicillin/streptomycin.
2) Allow cells to grow to confluency (typically 36-48 hours).
3) Switch cells to DMEM + 0.5% calf serum medium containing appropriate signaling agonists and or antagonists and controls. Continue growth of cells for 36-48 hrs.
4) Remove media and add 50 µl of lysis buffer to each well. Incubate with rocking for 45 minutes.
5) Transfer 10 µL of lysate to 96-well assay plate.
6) Add 50 µL of room temperature CDP-Star reagent to each lysate-containing well of the assay plate, and incubate for 15 minutes at room temperature in the dark.
7) Read plate on luminometer. One second or less integration time should be sufficient.

Notes

1) Cells may be seeded at higher density to reduce time to confluency.
2) Amount of lysate used in assay, as well as, amount of reagent are flexible but should not exceed a 1:2 ratio.
3) Incubation time before plate reading may have subtle effects on data and should be held constant for best comparison between experiments.