Detection of alkaline phosphatase (ALP) activity in marine fish cell lines

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PREAMBULE. Alkaline phosphatase activity should be measure using a plate reader equipped for 37ºC incubation and installed with kinetic software.

1. Remove culture medium and wash cells 3 times with PBS.
2. Add 100 µl of 0.1% triton X-100 (volume for 1 well of a 6-well plate) and disrupt cell layer using a cell scrapper.
3. Transfer cell extract into a 1.5-mL microcentrifuge tube and pellet cell debris by centrifugation (16,000 rcf for 1 min at 4ºC).
4. Transfer supernatant to a new tube and determine protein content (e.g., using Bradford reagent).
5. In each well of a 96 well plate, add 160 µl of reaction buffer and 20 µl of *p*-nitrophenyl phosphate (*p*-NPP) solution.
6. Place the plate into the plate reader pre-heated at 37ºC and measure absorbance at 405 nm for 10 min to determine basal activity.

   p-nitrophenol (p-NP) is produced from the breakdown of p-NPP by ALP and has its peak of absorption at 405 nm.
7. Add 20 µl of cell extract (i.e., supernatant) and measure absorbance at 405 nm for up to 1 h.
8. Subtract basal activity and determine ALP activity from initial slope.

   Convert absorbance into nmol of p-NP considering ε = 18.2 $mM^{-1}.cm^{-1}$ in 200 µl.
9. Normalize values of ALP activity using protein content (final unit = nmol $pNP.min^{-1}.mg\ protein^{-1}$).

Apparatus: a plate reader equipped for 37ºC incubation and installed with kinetic software.

Solutions: phosphate-buffered saline solution (PBS: 137 mM NaCl, 2.7 mM KCl, 15.8 mM Na_2HPO_4, 1.23 mM KH_2PO_4; pH 7.4); Reaction buffer (55 mM glycine, 0.55 mM $MgCl_2$, pH 10.5); p-nitrophenyl phosphate solution (5 mM p-NPP in reaction buffer).

Plasticware: 6-well cell culture dishes, serologic pipettes, cell scrappers and 96-well plates for plate reader.

All chemicals were purchased from Sigma-Aldrich, unless otherwise stated.

Additional information:

***Tiago DM, Cancela ML, Aureliano M, Laizé V** (2008) Vanadate proliferative and anti-mineralogenic effects are mediated by MAPK and PI-3K/Ras/Erk pathways in a fish chondrocyte cell line. FEBS Lett 582:1381-1385*

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