**PREAMBLE.** Alkaline phosphatase activity should be measure using a plate reader equipped for 37°C 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 4°C).
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 37°C 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 \( \varepsilon = 18.2 \text{ mM}^{-1} \text{ cm}^{-1} \) in 200 µl.
9. Normalize values of ALP activity using protein content (final unit = nmol pNP.min\(^{-1}\).mg protein\(^{-1}\)).

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**Apparatus:** a plate reader equipped for 37°C incubation and installed with kinetic software.

**Solutions:** phosphate-buffered saline solution (PBS: 137 mM NaCl, 2.7 mM KCl, 15.8 mM \( \text{Na}_2\text{HPO}_4 \), 1.23 mM \( \text{KH}_2\text{PO}_4 \); pH 7.4); Reaction buffer (55 mM glycine, 0.55 mM \( \text{MgC}_2 \), 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:**