Alkaline Phosphatase Activity (Colorimetric) Assay

Hongwei Cheng, 6-11-01; edited by TCH 8/15/01

1. Remove culture medium and carefully wash cells with PBS once.

2. Lyse cells with Lysis Solution (50mM Tris-HCl, pH 7.5, 0.5% Nonide P-40).

3. Prepare Sample Start Reagent: Combine 5 ml ALP Reagent B (by dissolving 2 tablets of ALP Reagent B in 5 ml deionized distilled water) with 25 ml ALP Reagent A (both from Sigma-Aldrich, Cat # DG1245-K).

4. Turn on spectrophotometer and set wavelength at 405nm.

5. Set absorbance reading to zero by using deionized distilled water as the blank reference.

6. Warm Sample Start Reagent to assay temperature (i.e., room temperature).

7. Add 1.0 ml of Sample Start Reagent to a 2 ml disposable cuvette. Add 50 μl of cell lysate sample and mix immediately by inversion. Record absorbance at 405nm as the reading of zero min.

8. Continue incubation at assay temperature and record absorbance after exactly 1, 2 and 3 minutes after reaction (Note: it is appropriate to record the reading at the end of the 3rd minute and divide the ABS by three).

9. Determine the mean absorbance change per minute (ΔA/min), and calculate ALP activity: ΔA/min $\times$ 1138 (IU).

NOTE:

1) If 48-well plates are used, lyse cells in 100 μl of Lysis Solution and use 50 μl for ALP enzymatic reactions.

2) Make sure cells are lysed completely by pipetting cell lysates up and down multiple times.

3) Sample Start Reagent is prepared freshly prior to use.

4) Enzymatic assays can be carried out at different temperature (e.g., 30 and 370C), but usually room temperature is just fine. However, if a higher assay temperature is chosen, a temperature conversion factor

should be used for activity calculation. Please consult with the manufacturer's manual for details.

Alternative Cell Lysis Solution:

20 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 1% Triton X-100.