

make the total Alkaline Phosphatase assay volume of 100 μ L/well. For a 384-well plate, add 25 μ L of Alkaline Phosphatase working solution into each well instead, for a total volume of 50 μ L/well.

3. Incubate the reaction for 30 to 60 minutes at room temperature, protected from light.
4. Add 50 μ L of Assay Buffer (Component D) into each well of Alkaline Phosphatase standard, blank control, and test samples with assay reaction mixture to make the total Alkaline Phosphatase assay volume of 150 μ L/well. For a 384-well plate, add 25 μ L of Assay Buffer (Component D) into each well, for a total volume of 75 μ L/well.
5. Monitor the luminescence increase with a standard luminescence plate reader within 1 to 10 minutes within 1 to 10 minutes.

Run alkaline phosphatase assay in cells:

1. Treat the cells as desired.
2. Remove the growth medium completely from the cell plate.

Note: It is important to remove the growth medium completely from the cell plate due to the interference of the growth medium with the phosphatase substrate.

3. Make 1:1 dilution of the 5 mL Alkaline Phosphatase working solution with 5 mL distilled H₂O.
4. Add 100 μ L (96-well plate) or 50 μ L (384-well plate) of 1:1 diluted Alkaline Phosphatase working solution into the cell wells.
5. Incubate the reaction at the desired temperature for 30 to 60 minutes, protected from light.
6. Add 50 μ L (96-well plate) or 25 μ L (384-well plate) of Assay Buffer (Component D) into the cell wells containing working solution.
7. Monitor the luminescence increase with a standard luminescence plate reader within 1 to 10 minutes.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RLU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the baseline corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate ALP samples. We recommend using the Online Linear Regression Calculator which can be found at:

<https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator>

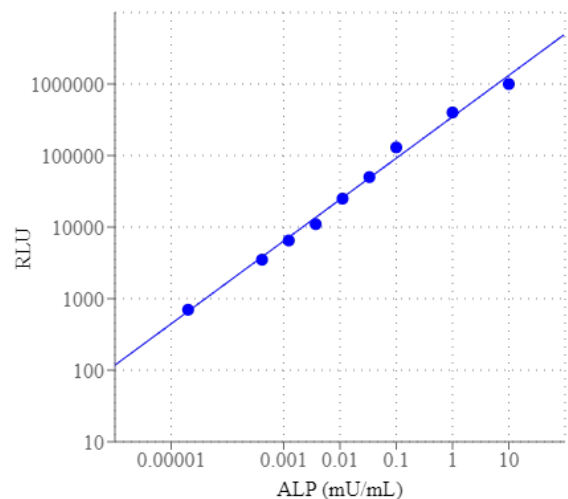


Figure 1. Alkaline phosphatase dose response was measured with Amplitude® Luminometric Alkaline Phosphatase Assay Kit in a white 96-well plate using a NovoStar microplate reader (BMG Labtech). As low as 0.001 mU/mL alkaline phosphatase can be detected with 20 minutes incubation (n=3).

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