Alkaline Phosphatase Activity

Version: 1
Edited by: Gary Cline, John Stack: Yale MMPC Analytical Core

Summary: Procedure to measure the amount of Alkaline Phosphatase activity. Alkaline Phosphatase (ALP) activity is measured from the hydrolysis of 4-nitrophenylphospate to 4-nitrophenyoxide ion (monitored at 405 nm) and phosphate.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Alkaline Phosphatase Reagent	Prolabs	R85120
Assayed Control Serum 1	Prolabs	R83082
Assayed Control Serum 2	Prolabs	R83083

Protocol:

Analysis by automated system Cobas Mira Plus.

1) Calibrate Cobas for Alkaline Phosphatase Activity analysis by running two assayed control serum.

2) Sample handling as performed by the Cobas Mira Plus.

- **a)** Pipette 3 µL of sample into a cuvette slot.
- **b)** Add 150 µL of Alkaline Phosphatase Reagent.
- **c)** Mixture is incubated at 37˚C and spun for 10 minutes.
- **d)** Absorbance is measured at 405 nm.

Reagent Preparation:

Alkaline Phosphatase Reagent: Add the appropriate amount of water (6.5mL) to the reagent bottle. Invert to mix, allowing 15 minutes for the reagent to settle.

Assayed Control Serum 1: Add the appropriate amount of water (6.5mL) to the chemical control bottle. Invert to mix, allowing 15 minutes for the reagent to settle.

Assayed Control Serum 2: Add the appropriate amount of water (6.5mL) to the chemical control bottle. Invert to mix, allowing 15 minutes for the reagent to settle.