

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

Summary: Procedure used to measure the Alanine Amino activity in blood, plasma, and serum. Alanine Amino (ALT) activity is measured by the enzymatically coupled reactions of ALT (to form pyruvate from alanine and α -ketoglutarate) and Lactate dehydrogenase (conversion of pyruvate to lactate with oxdiation of NADH to NAD). The rate of NAD formation is monitored by the change in absorbance at 340 nm.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Alanine Transaminase	Prolabs	R85122
Reagent		
Assayed Control Serum 1	Prolabs	R83082
Assayed Control Serum 2	Prolabs	R83083

Protocol: Analysis by automated system Cobas Mira Plus.

- 1) Calibrate Cobas for Alanine Transaminase Activity analysis by running two assayed control serum.
- 2) Sample handling as performed by the Cobas Mira Plus.
 - **a**) Pipette 16 μ L of sample into a cuvette slot.
 - **b**) Add 145 µL of Alanine Transaminase Reagent.
 - c) Mixture is incubated at 37°C and spun for 10 minutes.
 - d) Absorbance is measured at 340 nm.

Reagent Preparation:

Alkaline Transaminase 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.