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Yale - Alanine Aminotransferase

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Protocol status: Working

We use this protocol and it's working

Created: March 11, 2019

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Protocol Integer ID: 21276

Keywords: Alanine Amino activity, alanine aminotransferase summary, alanine amino activity in blood, alanine amino activity, alanine amino, dehydrogenase, alanine, lactate dehydrogenase, reactions of alt, conversion of pyruvate, oxidation of nadh, rate of nad formation, pyruvate, nad formation, nad, alt

Abstract

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 oxidation of NADH to NAD). The rate of NAD formation is monitored by the change in absorbance at 340 nm.

Materials

MATERIALS

 Assayed Control Serum 1 **Prolabs(cliniqa) Catalog #R83082**

 Assayed Control Serum 2 **Prolabs(cliniqa) Catalog #R83083**

 Alanine Transaminase Reagent **Prolabs(cliniqa) Catalog #R85122**

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.

Before start

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 μL 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.