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🌐 UC Davis - Alanine transaminase

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We use this protocol and it's working

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Abstract

Summary:

Alanine transaminase (ALT) also known as alanine aminotransferase or (sGPT) is a metabolic enzyme expressed primarily in the liver. Elevation of ALT levels is an indication of liver damage and has been associated with liver injury. ALT levels are monitored routinely in patients with liver diseases. ALT is also a very useful tool for preclinical investigation of experimental drug formulations and ALT levels are commonly used to monitor and attenuate the hepatotoxic effects of experimental drugs in rodents.

Materials

MATERIALS

| ⊗ ALT Kit **Bioo Scientific Catalog #3460-08** |

| ⊗ 0.5M NaOH |

| ⊗ Microplate |

| ⊗ Platereader |

Reagent Preparation:

ALT Reagent – Ready to use
DNPH Reagent – Ready to use

Standards – Prepare Pyruvate Standards by making serial dilutions of 70,32,15,7 U/l.

1   Add 10 µl of each sample or standard (in duplicate) to the microplate wells.

IMPORTANT: Make sure not to add any bubbles to the wells when dispensing reagents, this will interfere with reading in the platereader.

2   Add 50 µl of ALT Reagent Solution to the wells. Cover wells with the adhesive film and incubate at 37°C for 30 min.

3   Carefully remove adhesive film and add 50 µl DNPH Color Solution to the wells. Use second film to re-cover wells and incubate for 10 min at 37°C.

4   Remove adhesive and add 200 µl 0.5 M NaOH to each well. Read 510 nm absorbance in plate reader.

5   A standard curve can be constructed using the serially-diluted standards by plotting the average absorbance for each oxaloacetate standard against its concentration in U/l. Calculate unknowns from standard curve.