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UC Davis - Aspartate Aminotransferase

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We use this protocol and it's working

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Abstract

Summary:

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

Materials

MATERIALS

- AST Kit **Bioo Scientific Catalog #**5605-01
- 0.1 M HCl
- Microplate
- Platereader

Reagent Preparation:

AST Color Reagent – Reconstitute AST Color Reagent Mix with 30 mL of ddH_2O. Gently swirl to mix. Warm the AST Reagent Solution to 37°C for 10 minutes before use.

Standards – Prepare Oxaloacetate Standard by adding 250 µl of ddH_2O to the standard vial, then make serial dilutions of 400,200,100,50 U/l.

1 Add 5 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 AST Reagent Solution to the wells. Cover wells with the adhesive film and incubate at 37°C for 10 min.

3 Carefully remove adhesive film and add 50 µl AST Color Reagent 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 0.1 M HCl 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.