



ALT Protocol

Version: 1

Replaced by version

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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.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
ALT Kit	Bioo	3460-08
0.5M NaOH		
Microplate		
Platereader		

Protocol:

1. Add 10 μ l 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 μ l 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 μ l 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 μ l 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.

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.