
Determination of Alanine Aminotransferase (ALT/GPT) in clinical samples

Introduction

The enzyme Alanine Aminotransferase (ALT/GPT) reacts primarily with L-alanine and L-glutamate, but will also use aminobutyrate, ornithine or aspartate instead of alanine.



The liver is an important site for this metabolic reaction. Increased levels of ALT are associated with hepatitis and other liver disorders associated with hepatic necrosis as in cirrhosis, carcinoma and obstructive jaundice. Elevated levels occur in circulatory failure and extensive trauma. Expected values are up to 30U/l.

Principle

In the presence of alanine, pyruvate is formed by the action of ALT. This in turn is converted to lactate by the enzyme lactate dehydrogenase (LDH) in the presence of NADH, which is simultaneously converted to NAD and this is monitored at 340nm. As the action of LDH is inhibited by increasing lactate concentration which actually favours the reverse reaction and formation of pyruvate, the assay is monitored by following the initial rate of NAD formation over 3 minutes.



Method

The reagent can be prepared ready for use or obtained as a kit (Randox Labs, UK)

Pipette 1 ml of reagent containing:

LDH 1.25U/ml
NADH 0.18mmol/ml
2-oxoglutarate 15mmol/l
L-alanine 0.5 mmol/l
Tris buffer 100mmol/l pH 7.5

into each of 2 disposable cuvettes (80-2004-53).

Libra S21/S22 operation

- Select Applications (key 2) from the main menu
- Select Reaction rate
- Enter wavelength 340 and press OK (F3)
- Select time in mins
- Enter delay time of 1 and press OK (F3)
- Enter duration time at 4 and press OK (F3)
- Enter factor 1.746 to convert readings directly to IU/ml and press OK (F3)
 - To go back and change the parameters press Method (F1)
- A blank determination is necessary. The preparation is identical apart from omission of the sample and addition of an equivalent volume of water.
 - Add 0.1ml water (for the blank analysis) and mix.
- Insert blank and press green run key. This reference value is used for subsequent samples until changed
 - Read the absorbance rate of the blank over the period 1-4 minutes.
 - If there is any change in the blank value over the time period, the sample value should be appropriately corrected.
 - A single blank as above suffices for subsequent analyses in the same series.
- Insert samples as required and press the green run key.
 - Add 0.1 ml sample to the other reagent cuvette and mix.
 - Read the absorbance rate of the sample over the period 1-4 minutes.

The absorbance rate is shown as the slope of the graph over this time period.

Other information is displayed which, although not required for this calculation, is a check on the chemical stability of the assay. The assay is shown graphically as it proceeds, with absorbance difference and the line quality (a coefficient of determination of > 95 % is expected if the assay was carried out over a linear section).

Printouts may be obtained by setting up the printer options in System Utilities and Preferences (3) prints results. This is automatic with autoprint.

Additionally press **.** to print result if auto-print is off, or to re-print result if auto-print is on

The above procedure can be easily used with other instruments in the Libra range by using the concentration mode and the 1.746 factor.

Ordering Details

Libra S5	80-2115-00
Libra S11	80-2115-15
Libra S12	80-2115-10
Libra S21	80-2115-25
Libra S22	80-2115-20
Libra S32	80-2115-30

The reaction can be accelerated for increased sensitivity if warmed. For this purpose the Libra S21/S22 have the following accessories:

- 8 position water heated cell changer (80-2109-70) used with an external heating bath
- 6 position Peltier heated cell changer (80-2106-04) and Temperature Control Unit (80-2112-49)
- Single position water heated cell holder (80-2106-08) used with an external heating bath
- Single position electrical cell holder (80-2106-12), temperatures selectable from 25, 30 and 37°C
- Single position Peltier cell holder, temperatures selectable over the whole range from 20-49°C (80-2106-13).

The Sipper (80-2112-25) enables some automation of the analyses, and can be used together with a heated (not water heated) or non-heated single cell holder.