GPT (ALT)

CYPRESS DIAGNOSTICS

CE

IVD

NADH. UV. Kinetic.
According to IFCC
Liquid

Store at 2 - 8 °C

Configuration

REF	HBEL020	HBEL020A	HBEL020M
VOL	1 x 240 + 1 x 60 mL	6 x 120 + 3 x 60 mL	6 x 30 + 3 x 15 mL
Reagent 1	1 x 240 mL	6 x 120 mL	6 x 30 mL
Reagent 2	1 x 60 mL	3 x 60 mL	3 x 15 mL

Intended use

The Cypress Diagnostics GPT (ALT) kit is an in vitro diagnostic medical device intended to be used by healthcare professionals for the quantitative measurement of alanine aminotransferase (ALT/GPT) in human serum or plasma.
The measurement of alanine aminotransferase (ALT/GPT) is intended to be used for the screening for liver disorders, the aid in diagnosis of liver disease and the monitoring of the effectiveness of liver disease treatment.
For *in vitro* diagnostic use only. For professional use only.

Clinical significance

The enzyme alanine aminotransferase (ALT) or glutamate pyruvate transaminase (GPT) is widely reported in a variety of tissue sources. The major source of ALT is of hepatic origin and has led to the application of ALT determinations to the study of hepatic diseases. Elevated serum levels are found in hepatitis, cirrhosis and obstructive jaundice. When ALT is determined together with GOT (AST), in case of myocardial infarcts, the ALT levels will stay within the normal limits or only be slightly elevated in comparison to highly elevated AST levels.[1,4,5]
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

Principle

Kinetic determination of GPT (ALT) activity according to the following reaction:

$$\text{α-Ketoglutarate} + \text{L-Alanine} \xrightarrow{ALT} \text{Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + NADH + H^+ \xrightarrow{LDH} \text{Lactate} + NAD^+$$

The rate of NADH consumption is determined photometrically and is direct proportional to the GPT activity in the sample.[1]

Reagent composition

Reagent 1	**Buffer** TRIS buffer pH 7,5 (100 mmol/L) Lactate dehydrogenase (LDH) (2500 U/L) L-Alanine (600 mmol/L)
Reagent 2	**Substrate** NADH (1 mmol/L) α-ketoglutarate (100 mmol/L)

Precautions

- Reagent 1: Warning. H290: May be corrosive to metals. H319: Causes serious eye irritation. P234: Keep only in original container. P264: Wash hands and exposed skin thoroughly after handling. P280: Wear protective gloves/protective clothing/eye protection/face protection. P501: Dispose of contents/container in an appropriate container observing applicable local regulations.
- EUH032: Contact with acids liberates very toxic gas.
- All body fluid samples should be considered potentially infectious materials and the appropriate precautions should be taken. Wear personal protective equipment such as gloves, safety glasses, lab coats or aprons when working with possible biohazard contaminants.
- Use Good Laboratory Practices (GLP) when handling this product.
- Please refer to the MSDS, available on our website, for further information.

Preparation

Mix 4 volumes of R1 (Buffer) with 1 volume of R2 (Substrate). The stability of the working reagent is 24 hours at 15-25 °C or 4 weeks at 2-8 °C.

Storage, stability and disposal

All the components of the kit are stable up to the date of expiration as specified on the label, when stored tightly closed, protected from light and contaminations prevented during their use. Storage temperature for this kit is 2 - 8 °C.
The reagent should be a clear solution. If turbidity or precipitation has occurred or if blank absorbance at 340 nm < 1,00, the reagent should be discarded.
Do not use the product if deterioration or contamination is suspected or beyond the expiration date or open container stability period. Dispose unused or deteriorated product and waste in compliance with local regulations.

Additional material required but not provided

- Spectrophotometer or colorimeter. Minimum analyzer specifications:
  Measuring at 340 nm
  Linear measuring range: 0 - 2 AU
- Thermostatic bath at 25 °C, 30 °C or 37 °C (± 0,1 °C).
- Cuvettes, matching the analyzer used (1,0 cm light path)
- General laboratory equipment

Samples

Serum or plasma: stability 1 day at 2-8 °C.[6]
Fasting of at least 12 hours is recommended before sample collection.

Procedure

Make sure the reagents and samples are at room temperature.
1. Wavelength 340 nm; Temperature 25, 30, 37 °C; Cuvette (1 cm light path).
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:

For Sample	100 µL Sample + 1,00 mL Working Reagent
Mix and wait 1 min. Read initial absorbance (A), start the stopwatch and read absorbances every minute for 3 min. Calculate the difference between the absorbances and the average absorbance differences per minute (ΔA/min).	

Calculation

GPT (ALT) (U/L)= ΔA/min x 1750 [Note 4]

One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per liter of sample (U/L).

Conversion Factor: µkat/L = 0,0167 x U/L

Temperature conversion factors
To correct results to other temperatures, multiply by:

Assay Temperature	Desired Temperature		
	25 °C	30 °C	37 °C
25 °C	1,00	1,32	1,82
30 °C	0,76	1,00	1,39
37 °C	0,55	0,72	1,00

Quality control

Control sera are recommended for the qualification of the reagents and to monitor the performance of assay procedures. Next to legal requirements, we recommend to perform Quality Control on a daily basis and after each calibration.
Use Biochemistry Normal and Pathological Controls (HBC01, HBC02). If other controls (not manufactured by Cypress Diagnostics) are used, they have to be validated by the user as they can vary. Prepare and measure these controls the same as samples. Measure at least one replicate per control.
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems. Do not continue testing as the results will be invalid. Each laboratory should establish its own QC scheme and corrective actions if controls do not meet the acceptable tolerances.

Reference values[4,5]

Men - 25 °C	< 22 U/L (< 0,37 µkat/L)
Men - 30 °C	< 29 U/L (< 0,48 µkat/L)
Men - 37 °C	< 40 U/L (< 0,67 µkat/L)
Women - 25 °C	< 18 U/L (< 0,30 µkat/L)
Women - 30 °C	< 22 U/L (< 0,37 µkat/L)
Women - 37 °C	< 32 U/L (< 0,53 µkat/L)

Normal newborns have been reported to show a reference range of up to double the adult, attributed to the neonate's hepatocytes. These values decline to adult levels at approximately the age of 3 months.
These values are for orientation purpose. Each laboratory should establish its own reference range.

Performance characteristics

Measuring range: from 3 U/L (detection limit) to 260 U/L (linearity limit). If the obtained results are greater than 260 U/L, dilute the sample 1:10 with saline solution, repeat the determination, and multiply the result by factor 10.

Precision:

	intra-assay (n=20)		inter-assay (n=20)	
Mean (U/L)	43,4	125	44,7	127
SD	0,41	0,67	1,03	2,71
CV (%)	0,94	0,53	2,30	2,13

Sensitivity: 1 U/L = 0,00055 ΔA/min

Accuracy: Results obtained using CYPRESS DIAGNOSTICS reagents did not show systematic differences when compared with other commercial reagents.
The results of the performance characteristics depend on the analyzer used.

Cypress Diagnostics: Nijverheidsstraat 8 • 2235 Hulshout • Belgium
www.diagnostics.be • Tel: ++ 32 15 67 67 68 • e-mail: cypress@diagnostics.be

Interferences

Anticoagulants currently in use like heparin, EDTA, oxalate and fluoride do not affect the results. No hemoglobin interference up to 42 mg/dL. A list of drugs and other interfering substances with ALT determination has been reported by Young et al.[2,3] Hemolysis and lipemia interfere with the assay.[6,7]

Notes

1. **For best use of this kit on a Cypress Diagnostics analyzer, we kindly advise to follow the application sheets of the respective analyzer. Please log in to our website (www.diagnostics.be) as a registered user to download the latest application sheets, which are located under the section of the corresponding analyzer. Compatible Cypress analyzers: CYANSmart, CYANStart, CYANExpert 130, CYANVision.**
2. **In case other instruments (not manufactured by Cypress Diagnostics) are used, the laboratory is responsible to validate the reagents in this kit on those analyzers before testing patient samples.**
3. **For this kit, application sheets for the following Mindray analyzers are available (see website): BS-120, BS-200, BS-200E.**
4. **The factor is based on official IFCC conditions. For more accurate results, we recommend to use a serum based Biochemistry Calibrator (HBC03) instead of a factor.**

Bibliography

1. **Murray R. Aspartate aminotransferase. Kaplan A et al. Clin Chem The C.V. Mosby CO. St.Louis. Toronto. Princeton. 1984; 1088-1090.**
2. **Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed. AACC Press 1995.**
3. **Young DS. Effects of diseases on Clincal Lab. Tests, 4th ed. AACC 2001.**
4. **Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed. AACC 1999**
5. **Tietz N W et al. Clinical Guide to Laboratory tests, 3rd ed. AACC 1995.**
6. **Rifae N. Horvath A.R. and Wittwer C.T. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 6th ed. AACC Press 2018.**
7. **Nikolac N. Biochemia Medica. 2014, 24 : 57-67.**

Notice: Any serious incident that has occurred in relation to the device shall be reported to Cypress Diagnostics and the competent authority of the Member State in which the user and/or patient is established.

2022-02, Rev. 5.0

Cypress Diagnostics: Nijverheidsstraat 8 • 2235 Hulshout • Belgium
www.diagnostics.be • Tel: ++ 32 15 67 67 68 • e-mail: cypress@diagnostics.be