


GPT REITMAN-FRANKEL COLORIMETRIC METHOD

IVD For in vitro diagnostic use only.

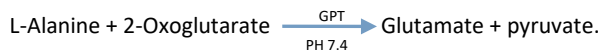
2°C  8°C
Store at 2-8°C

INTENDED USE

For the determination of GPT concentration in human serum.

PRINCIPLE

Alanine aminotransferase (GPT) catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of glutamate and pyruvate.



The transaminase activity is proportional to the amount of pyruvate formed over a definite period of time and is measured by the reaction with 2,4-dinitrophenylhydrazine (DNPH) and measurement of the color formed in an alkaline solution.

REAGENTS

Materials Provided

R1 GPT SUBSTRATE	Phosphate buffer 150 mmol/L PH 7.4 L-alanine 200 mmol/L ketoglutarate 2 mmol/L
R2 DNPH	2,4-Dinitrophenylhydrazine 1 mmol/L Color developer
R3 4N NaOH (10x)	Sodium hydroxide 4 mol/L
CAL	Pyruvic standard 1.8 mmol/L Secondary standard

EQUIPMENTS NEEDED BUT NOT PROVIDED

- Photometer for measurements at 505 nm ± 15 nm.
- Thermostatic water bath set at 37°C (± 1°C).
- Stopwatch.
- Pipettes of 5.0 mL, 1.0 mL and 0.1 mL.
- Glass tubes.

SAMPLES

Serum, free of hemolysis.

Transaminases are stable in serum 24 hours at room temperature and for 1 week at 2-8°C.

REAGENT PREPARATION

The substrates, standard and color developer are ready to use.

Working 0.4 N NaOH solution. Dilute 10x 4N NaOH reagent to 1x 4N NaOH in Distilled water. For example for each 9 ml of distilled water add 1 ml 10x 4N NaOH reagent to equal final volume of 10 ml.

Mix again and store in a well capped polyethylene bottle at room temperature

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C.

PROCEDURE

1. Bring reagents and samples at room temperature.
2. Pipette into labeled tubes:

Tubes	Blank	GPT
GPT substrate	0.5 mL	0.5 mL

3. Warm to 37°C into the bath for 5 min then add:

Serum	-	100 µl
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4. Mix then Return to bath at 37°C for 30 min.

5. Add R2 DNPH:

DNPH	0.5 mL	0.5 mL
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6. Mix. Stand for 20 min at room temperature.

7. Add NaOH:

NaOH 0.4N	5.0 mL	5.0 mL
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8. Invert to mix. Stand for 5 min. at room temperature.

9. Read the absorbances (A) of the samples against water blank. (The color is stable for at least 1 hour).

CALCULATIONS

From absorbencies, read units of GPT from corresponding curves. For activities higher than 100 WU (GPT) repeat the test diluting the sample 1:10 with saline and assayed again. Multiply the results by 10.

UNITS

The conversion of colorimetric units into UV units obtained by a kinetic optimized method (IFCC, 1985) cannot take place by the use of a factor as in the classic UV Karmen procedure (1995).

REFERENCE VALUES

GPT/ALT = 5-30 WU/ml.

Results between 30-40 WU (GPT) are considered borderline values.

QUALITY CONTROL

To ensure adequate quality control (QC) with this method, the inclusion in each run of controls (normal and abnormal) with assayed values will help to find the equivalence between units.

INTERFERENCES

- Samples with patients under hemodialysis, severe vitamin B deficiency or with related pathologies, lead to an underestimation of GPT values.
- Highly elevated levels of hemolysis are not suitable for testing.
- Other drugs and substances may affect the GPT values.

CLINICAL SIGNIFICANCE

The group of enzymes called transaminase exists in tissues of many organs. Necrotic activity in these organs causes a release of abnormal quantities of enzyme into the blood.

The liver is especially rich in GPT. This enzyme measurement is used primarily as a test for hepatitis. With infectious hepatitis the GPT activity in serum is greater than of GOT, but both activities usually are increased. Thus, generally speaking, the GPT value is useful in diagnosing infectious hepatitis. Neither test is specific.

NOTES

1. The tests may be read against water set at zero absorbance. However, the technic of running a reagent blank and setting this at 0.250 A helps to compensate for small changes that might occur in the reagents or instrument.
2. Dilutions of serum do not always give values in exact proportion to the magnitude of the dilution.
3. This standard, when used as described gives a curve which agrees more closely with the reference method than does the 2 mmol/L original standard recommended by Reitman and Frankel.

CALIBRATION SETTING

1. Pipette (mL) into labelled tubes:

Tubes	PYRUVIC STD	GPT SUBSTRATE	H ₂ O	WU/mL GPT
1	-	1.0	0.2	0
2	0.1	0.9	0.2	28
3	0.2	0.8	0.2	57
4	0.3	0.7	0.2	97
5	0.4	0.6	0.2	-

2. Add to each tube 1.0 mL of DNPH. Mix. Stand for 20 minutes at room temperature.
3. Add to each tube 10.0 mL of 0.4N NaOH. Mix. Stand for at least 15 minutes.
4. Blank the instrument with distilled water.

- Using linear graph paper plot the units of activity shown above against their respective absorbances (A) at 505 nm. Check the curve periodically (Note 3).

REFERENCES

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- Bergmeyer, H.U., HØrder, M., Rej, R. Approved Recommendation (1985) on IFCC Methods for the Measurement of Catalytic Concentration of Enzymes. Part 2. IFCC Method for Aspartate Aminotransferase, J. Clin. Chem. Clin. Biochem. 24, 497-510.
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ATLAS Medical

Ludwig-Erhard Ring 3

15827 Blankenfelde-Mahlow

















Germany

Tel: +49 - 33708 – 3550 30

Email: Info@atlas-medical.com

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 REF	Catalogue Number		Temperature limit
 IVD	<i>In Vitro</i> diagnostic medical device		Caution
 Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
 LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry