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GOT (AST)
mod. IFCC

Diagnostic reagent for quantitative in vitro determination of GOT (AST) in human serum or plasma on photometric systems

REF	Kit Size	Configuration
D03115B	1 x 1.25 L	1 x 1 L R1 + 1 x 0.25 L R2
D94610	5 x 100 mL	4 x 100 mL R1 + 1 x 100 mL R2
D98616	5 x 50 mL	4 x 50 mL R1 + 1 x 50 mL R2
D00678	5 x 25 mL	4 x 25 mL R1 + 1 x 25 mL R2
D98617	5 x 10 mL	4 x 10 mL R1 + 1 x 10 mL R2
D72911	10 x 50 mL	10 x 40 mL R1 + 4 x 25 mL R2
D0427917	5 x 62.5 mL	4 x 62.5mL R1 + 1 x 62.5mL R2
DA0829	5 x 50 mL	5 x 40 mL R1 + 5 x 10 mL R2
DT1029	4 x 62.5 mL	4 x 50 mL R1 + 4 x 12.5mL R2
DK0728	5 x 50 mL	4 x 50 mL R1 + 1 x 50 mL R2
DE1829	4 x 62.5 mL	4 x 50 mL R1 + 4 x 12.5 mL R2
DB20322	4 x 62.5 mL	4 x 50 mL R1 + 4 x 12.5 mL R2

Additionally available:

D98485	5 x 3 mL	Calibrator	Diacal Auto
D98485SV	1 x 3 mL	Calibrator	Diacal Auto
D98481	12 x 5 mL	Control normal	Diacon N
D14481	5 x 5 mL	Control normal	Diacon N
D98481SV	1 x 5 mL	Control normal	Diacon N
D98482	12 x 5 mL	Control abnormal	Diacon P
D14482	5 x 5 mL	Control abnormal	Diacon P
D98482SV	1 x 5 mL	Control abnormal	Diacon P

For professional in vitro diagnostic use only.

GENERAL INFORMATION

Method	UV, kinetic, decreasing reaction, modified IFCC
Shelf life	15 months from production date
Storage	2 – 8 °C
Wavelength	340 nm, Hg 334 nm, Hg 365 nm
Optical path	1 cm
Temperature	37°C
Sample	Serum, EDTA-plasma, heparinized plasma

INTENDED USE

Diagnostic reagent for quantitative in vitro determination of GOT (AST) in human serum or plasma on photometric systems.

DIAGNOSTIC SIGNIFICANCE [1, 2]

Alanine Aminotransferase (ALAT/ALT), also called Glutamic Pyruvic Transaminase (GPT) and Aspartate Aminotransferase (ASAT/AST), formerly called Glutamic Oxalacetic Transaminase (GOT) are the most important representatives of a group of enzymes, the aminotransferases or transaminases, which catalyse the conversion of α-keto acids into amino acids by transfer of amino groups.
As liver specific enzyme GPT is only significantly elevated in hepatobiliary diseases. Increased GOT levels, however, can occur in connection with damages of heart or skeletal muscle as well as of liver parenchyma. Parallel measurement of GPT and GOT is therefore applied to distinguish liver from heart or skeletal muscle damages. The GOT/GPT ratio is used for differential diagnosis in liver diseases. While ratios < 1 indicate mild liver damage, ratios > 1 are associated with severe, often chronic liver diseases.

TEST PRINCIPLE

L-Aspartate + 2-Oxoglutarate $\xrightarrow{GOT}$ Oxaloacetate + L-Glutamate

Oxaloacetate + NADH + H^+ $\xrightarrow{MDH}$ L-Malate + NAD^+

NADH is oxidized to NAD^+, the resulting decrease in absorbance at 340 nm is directly proportional to the activity of GOT in the sample.
This is a modified formulation for the assay of GOT, as recommended by the IFCC (International Federation of Clinical Chemistry). The IFCC reference method includes pyridoxal-5'-phosphate (P-5-P). P-5-P serves as coenzyme in AA transfer and stabilizes the activity of transaminases. Therefore, addition of P-5-P avoids falsely low values in samples containing insufficient endogenous P-5-P, e.g. from patients with myocardial infarction, liver disease and intensive care patients [1, 3].

AA = Amino Acid
GOT = Glutamate Oxaloacetate Transaminase
MDH = Malate Dehydrogenase
NAD^+ = Nicotinamide Adenine Dinucleotide
NADH = reduced NAD
LDH = Lactate Dehydrogenase

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION	
Reagent 1		
Tris, pH 7.8	110	mmol/L
L-Aspartate	340	mmol/L
MDH	0.5	kU/L
LDH	1.1	kU/L
Reagent 2		
2-Oxoglutarate	85	mmol/L
NADH	≥ 1	mmol/L

MATERIAL REQUIRED BUT NOT PROVIDED

- NaCl solution (9 g/L).
- Clinical chemistry analyser.
- Dialab Pyridoxal-5'-Phosphate in case of determination with P-5-P.

REAGENT PREPARATION

Substrate Start:
Reagents are ready to use.

Sample Start:
Mix 4 parts of Reagent 1 with 1 part of Reagent 2.
(= working reagent)

STORAGE AND STABILITY

Conditions: Protect from light.
Avoid contamination.
Close immediately after use.
Do not freeze the reagents!

Substrate Start:
Storage: at 2 – 8 °C
Stability: up to the expiration date

Sample Start (Working Reagent):
Stability: at 2 – 8 °C 4 weeks
at 15 – 25 °C 5 days

The working reagent must be protected from light!

WARNINGS AND PRECAUTIONS

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Reagent 1 contains animal material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practices.
3. In very rare cases, samples of patients with gammopathy might give falsified results [4].
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
5. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
6. For professional use only!

SPECIMEN COLLECTION AND STORAGE

Stability [5]:
In serum/plasma at 20 – 25 °C 4 days
at 4 – 8 °C 7 days
at - 20 °C 3 months
Discard contaminated specimens. Freeze only once!

TEST PROCEDURE

Bring reagents and samples to room temperature.

Note: If pyridoxal phosphate (P-5-P) is used, please consult instruction insert for P-5-P before performing test (for Substrate Start only).

Substrate Start

Pipette into test tubes	37°C
Reagent 1	1000 µl
Sample	100 µl
Mix. Incubate for 5 min. Then add:	
Reagent 2	250 µl
Mix. Read initial absorbance against air after 1 min. and start a timer. Read absorbance again after exactly 1, 2 and 3 min.	

Sample Start (do not use sample start with P-5-P)

Pipette into test tubes	37°C
Working reagent	1000 µl
Sample	100 µl
Mix. Read initial absorbance against air after 1 min. and start a timer. Read absorbance again after exactly 1, 2 and 3 min.	

Automation

Special adaptations for automated analysers can be made on request.

INTERPRETATION OF RESULTS

Calculation
With factor (light path 1 cm)**:**
From absorbance readings calculate ΔA/min and multiply by the corresponding factor:

GOT [U/L] = ΔA/min x factor

Factors (37 °C):

Substrate Start:

Factor at 340 nm	2143
Factor at 334 nm	2184
Factor at 365 nm	3971

Sample Start:

Factor at 340 nm	1745
Factor at 334 nm	1780
Factor at 365 nm	3235

With calibrator:

$$GOT\ [U/L] = \frac{\Delta A/min\ Sample}{\Delta A/min\ Calibrator} \times Conc.\ Calibrator\ [U/L]$$

Unit Conversion
GOT [U/L] x 0.0167 = GOT [µkat/L]

QUALITY CONTROL AND CALIBRATION

All control sera with GOT values determined by this method can be used.
We recommend the Dialab serum controls **Diacon N** (control serum with values in the normal range) and **Diacon P** (control serum with values in the abnormal range).
Each laboratory should establish corrective action in case of deviations in control recovery.

Calibration
The use of a GOT Calibrator is optional.
We recommend the Dialab multi calibration serum **Diacal Auto**.

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE

On automated systems the test is suitable for the determination of GOT activities up to 700 U/L.
In case of manual procedure, the test is suitable for GOT activities which correspond to a maximum ΔA/min = 0.16 at 340 nm and 334 nm or 0.08 at 365 nm.
Above this concentration the samples should be diluted 1+9 with NaCl solution (9 g/L) and the results multiplied by 10.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 4 U/L.

PRECISION (at 37 °C)

Without P-5-P

Intra-assay, n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	25.1	0.82	3.25
Sample 2	51.3	1.57	3.06
Sample 3	116	0.90	0.77

Inter-assay, n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	25.7	1.13	4.40
Sample 2	48.6	0.67	1.38
Sample 3	115	0.80	0.69

With P-5-P

Intra-assay, n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	43.6	1.10	2.51
Sample 2	74.5	1.79	2.41
Sample 3	174	3.18	1.83

Inter-assay, n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	44.0	1.59	3.61
Sample 2	77.0	3.05	3.97
Sample 3	187	3.37	1.80

SPECIFICITY/INTERFERENCES

no interference up to:

Ascorbic acid	30 mg/dL
Bilirubin	40 mg/dL
Triglycerides	2000 mg/dL

The presence of hemoglobin in serum indicates destruction of erythrocytes with release of GOT, thus producing high interference.
For further information on interfering substances refer to Young DS [6].

METHOD COMPARISON

With P-5-P
A comparison between Dialab GOT (AST) (y) with P-5-P and a commercially available test (x) using 107 samples gave following results: y = 0.961 x + 4.227; r= 0.998.

Without P-5-P
A comparison between Dialab GOT (AST) without P-5-P (y) and a commercially available test (x) using 105 samples gave following results: y = 0.967 x – 0.174 U/l; r= 1.000.

TRACEABILITY

This method has been standardized against the original IFCC formulation.

EXPECTED VALUES*

With P-5-P:		U/L	µkat/L
Women [7]		< 31	< 0.52
Men [7]		< 35	< 0.58
Children [1]	1 – 3 year(s)	< 50	< 0.83
	4 – 6 years	< 45	< 0.75
	7 – 9 years	< 40	< 0.67
	10 – 12 years	< 40	< 0.67
	13 – 15 years	< 35	< 0.58
	16 – 18 years	< 35	< 0.58

Without P-5-P:		
Women [8, 9]	< 31 U/L	< 0.52 µkat/L
Men [8, 9]	< 35 U/L	< 0.58 µkat/L

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

LIMITATIONS

- Eventual GOT (AST), mod. IFCC carry-over to reagent Protein Total in Urine/CSF (Pyrogallol red). The actual carry-over depends on the analyser.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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