

Liquid Reagents – ready to use

GOT (AST)

(Glutamate Oxaloacetate Transaminase)

Modified IFCC

2 Reagents

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

Ref.No.	Kit Size	Content
DIA010122	5 x 25 ml	4x25 ml R1+1x25 ml R2
DIA010123	5 x 50 ml	4x50 ml R1+1x50 ml R2
DIA010124	5 x 100 ml	4x100 ml R1+1x100 ml R2

Additionally offered:

DIA040012	1 x 3 mL	Diacal Auto (Calibrator)
DIA030012	1 x 5 mL	Diacon N (Control Normal)
DIA030022	1 x 5 mL	Diacon P (Control Abnormal)

TEST PARAMETERS

Method:	UV, kinetic, decreasing reaction, modified IFCC
Wavelength:	Hg 334 nm, Hg 365 nm, 340 nm
Temperature:	25°C, 30°C, 37°C
Sample:	Serum, EDTA-plasma, heparinized plasma
Linearity:	up to 940 U/L on Hitachi 911
Sensitivity:	The lower limit of detection is 2 U/L

REAGENT COMPOSITION

COMPONENTS	FINAL CONCENTRATION	
Reagent 1:		
Tris, pH 7.8	80	mmol/L
L-Aspartate	240	mmol/L
MDH	≥ 600	U/L
LDH	≥ 1200	U/L
Reagent 2:		
2-Oxoglutarate	16	mmol/L
NADH	≥ 0.18	mmol/L

REAGENT PREPARATION

Substrate Start:

Reagents are ready for use.

Sample Start:

Mix 4 parts of Reagent 1 with 1 part of Reagent 2.
(= Working Reagent)

REAGENT STABILITY AND STORAGE

Conditions: protect from light. close immediately after use. Do not freeze the reagents!

Substrate Start:

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

Sample Start (Working Reagent):

Stability: at 2 – 8°C 4 weeks
at 15 – 25°C 5 days

Minimum allowable absorbance of the Working Reagent measured at 340 nm against water as reference is 1.6.

SAMPLE STABILITY AND STORAGE

Loss of activity: at 2 - 8°C < 8 % within 3 days
at 15 - 25 °C < 10 % within 3 days

Stability: at -20 °C at least 3 months

Discard contaminated specimens.

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
General laboratory equipment

INTERFERING SUBSTANCES

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.

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

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

Substrate Start

Pipette into test tubes	25°C, 30°C	37°C
Reagent 1	1000 µl	1000 µl
Sample	200 µl	100 µl
Mix. Incubate for approximately 5 min. Then add:		
Reagent 2	250 µl	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 PP)

Pipette into test tubes	25°C, 30°C	37°C
Working reagent for sample start	1000 µl	1000 µl
Sample	200 µl	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.		

CALCULATION (light path 1 cm)

GOT (IU/L) = ΔA/min x Factor

Factors:

Substrate Start:	25° or 30°C	37°C
Factor at 340 nm	1151	2143
Factor at 334 nm	1173	2184
Factor at 365 nm	2132	3971
Sample Start:	25° or 30°C	37°C
Factor at 340 nm	952	1745
Factor at 334 nm	971	1780
Factor at 365 nm	1765	3235

UNIT CONVERSION

U/L x 0.01667 = µkatal/L

REFERENCE RANGE* [U/L]

without addition of pyridoxal phosphate:

	25°C	30°C	37°C
Males:	< 18	< 25	< 37
Females:	< 15	< 21	< 31
with addition of pyridoxal phosphate:			
		30°C	37°C
Males:		7 – 34	10 – 50
Females:		7 – 24	10 – 35

* It is recommended that each laboratory establishes its own normal range.

TEST PRINCIPLE

NADH is oxidized to NAD⁺, the resulting decrease in absorbance at 340 nm is directly proportional to the activity of GOT in the sample.

L-Aspartate + 2-Oxoglutarate $\xrightarrow{\text{GOT}}$ Oxaloacetate + L-Glutamate
Oxaloacetate + NADH + H⁺ $\xrightarrow{\text{MDH}}$ L-Malate + NAD⁺

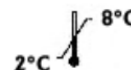
This is a modified formulation for the assay of GOT, as recommended by the IFCC (International Federation of Clinical Chemistry). The IFCC reference method includes pyridoxal phosphate (PP). PP functions as coenzyme in AA transfer, therefore addition of PP results in increased enzyme activity. It avoids falsely low values in samples containing insufficient endogenous PP, e.g. from patients with myocardial infarction, liver disease and intensive care patients.

ABBREVIATIONS

AA	= Amino Acid
GOT	= Glutamate Oxaloacetate Transaminase
MDH	= Malate Dehydrogenase



NAD⁺ = Nicotinamide Adenine Dinucleotide
 NADH = reduced NAD
 LDH = Lactat Dehydrogenase



PERFORMANCE CHARACTERISTICS

LINEARITY

The assay is linear up to a $\Delta A/\text{min} = 0.16$ at 340nm and 334nm or 0.08 at 365nm.

Above this concentration dilute the sample with NaCl solution (9 g/L sodium chloride in dist. water) and reassay multiplying the result by the dilution factor.

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

METHOD COMPARISON

Without P-5-P

A comparison between Diagnostica GOT (AST) without P-5-P (y) and a commercially available test (x) using 78 samples gave following results: $y = 0.98x - 0.51$ U/l; $r = 0.999$.

With P-5-P

A comparison between Diagnostica GOT (AST) (y) with P-5-P and a commercially available test (x) using 80 samples gave following results: $y = 0.97x - 0.79$; $r = 0.995$.

QUALITY CONTROL

All control sera with GOT values determined by this method can be used.

We recommend:

DIA030012	1 x 5 ml	DIACON N	Assayed Control Serum Normal
DIA030022	1 x 5 ml	DIACON P	Assayed Control Serum Abnormal

CALIBRATION

The use of a GOT Calibrator is optional.

We recommend:

DIA040012	1 x 3 ml	DIACAL	Assayed Multi Calibration Serum
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AUTOMATION

Special adaptations for automated analyzers can be made on request.

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. Take the necessary precautions for the use of laboratory reagents.

WASTE MANAGEMENT

Please refer to local legal requirements.

