Pointe
AST Reagent Set

HORIBA Instruments Incorporated
5449 Research Drive, Canton, MI 48188
Phone: 734-487-8300; (800) 445-9853

Intended Use
For the quantitative determination of Aspartate Aminotransferase (AST) in human serum using the Yumizen C560 analyzer. **Rx Only.**

Clinical Significance
AST is widely distributed in tissues with the highest concentrations found in the liver, heart, skeletal muscle and kidneys. Diseases involving any of these tissues can lead to elevated levels of AST in serum. Following myocardial infarction, AST levels are elevated and reach a peak after 48 to 60 hours.
Hepatobiliary diseases such as cirrhosis, metastatic carcinoma and viral hepatitis can show increased levels of AST. Other disorders which can lead to an elevated level of AST are muscular dystrophy, dermatomyositis, acute pancreatitis and infectious mononucleosis.[1]

Method History
Karmen[2] developed a kinetic assay procedure in 1955 which was based upon the use of malate dehydrogenase and NADH. Optimized procedures were presented by Henry[3] in 1960 and Amador and Wacker[4] in 1962. These modifications increased accuracy and lowered the effect of interfering substances. The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology[5] published a recommended method based on optimized modifications in 1974. In 1976, the Expert Panel on Enzymes of the International Federation of Clinical Chemistry (IFCC)[6] proposed the addition of pyridoxal-5-phosphate to the reaction mixture to ensure maximum activity. The IFCC[7] published a recommended method that included P-5-P in 1978. The present method is based on IFCC recommendations but does not contain P-5-P since most specimens contain adequate amounts of this cofactor for full recovery of AST activity.[8,9,10]

Principle
$$\text{L-Aspartate} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{AST}} \text{Oxalacetate} + \text{L-Glutamate}$$

$$\text{Oxalacetate} + \text{NADH} + \text{H+} \xrightarrow{\text{MDH}} \text{L-Malate} + \text{NAD}^{+} + \text{H}_2\text{O}$$

Aspartate aminotransferase (AST) catalyzes the transfer of the amino group from L-aspartate to α-Ketoglutarate to yield oxalacetate and L-glutamate. The oxalacetate undergoes reduction with simultaneous oxidation of NADH to NAD in the malate dehydrogenase (MDH) catalyzed indicator reaction. The resulting rate of decrease in absorbance at 340nm is directly proportional to the AST activity. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate which is normally present in serum.

Reagents
After combining R1 and R2, the reagent contains: L-aspartic acid 200mM, α-ketoglutaric acid 11mM, LDH (microbial) > 1000U/L, MDH (microbial) ≥800U/L, NADH >0.18mM, buffer, sodium azide 0.28%, stabilizers.

Reagent Preparation
The reagents are ready to use.

Reagent Storage
Store the reagents at 2-8°C. The reagent is stable until the expiration date appearing on the label when stored as directed. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.

Reagent Deterioration
Do not use reagent if:
1. The initial absorbance at 340nm is below 0.800.
2. The reagent fails to meet stated parameters of performance.

Precautions and Hazards
1. This reagent set is for *in vitro* diagnostic use only.
2. The reagent contains sodium azide (0.28%) as a preservative. Do not ingest. May react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build up.

Hazards:


Signal Word: Warning

R1: Hazard Classifications: Specific Target Organ Toxicity, Single Exposure; Respiratory System (Category 3)
Hazard Statements: H335: May cause respiratory irritation.
Precautionary Statements: **Prevention:** P261 Avoid breathing dust/fume/gas/mist/vapors/spray. P271 Use only in a well-ventilated area. **Response:** P312 Call a POISON CENTER or doctor/physician if you feel unwell. P304 + P340 IF INHALED: Remove victim to fresh air and Keep at rest in a position comfortable for breathing. **Storage:** P403 + P233 Store in a well-ventilated place. Keep container tightly closed. **Disposal:** P501: Dispose of contents into sewer system after diluting with large volumes of water, if in accordance with local regulations.

Signal Word: Warning

R2: Hazard Classifications: Acute Toxicity, Dermal (Category 4),
Hazard Statements: H312: Harmful in contact with skin
Precautionary Statements: **Prevention:** P280 Wear protective gloves/protective clothing/eye protection/face protection. P312 Call a POISON CENTER or doctor/physician if you feel unwell. P363 Wash contaminated clothing before reuse. P302 + P352 IF ON SKIN: wash with plenty of soap and water. **Storage: Disposal:** P501: Dispose of contents into sewer system after diluting with large volumes of water, if in accordance with local regulations. **Refer to the Safety Data Sheet for this product (SDS-A7561) available by calling 1-734-487-8300.**

Specimen Collection and Storage[11]
1. Non-hemolyzed serum is recommended. Red cells contain AST which can give falsely elevated results.
2. AST in serum is reported stable for ten days when refrigerated (2-8°C), two weeks when frozen (-20°C), and four days when stored at room temperature (15-30°C).

Interferences
1. A number of drugs and substances affect AST activity. See Young, et al.[12]
2. Patients with severe vitamin B6 deficiency could have a decreased recovery of AST, presumably due to a lack of pyridoxal phosphate.[13]
3. Bilirubin to at least 18 mg/dl, and hemoglobin to at least 300 mg/dl, have been found to have a negligible effect on this procedure.

Materials Provided
AST (SGOT) Reagents R1 and R2

Materials Required but not Provided
1. Yumizen C560 Analyzer
2. Yumizen C560 Operation manual
3. Chemistry control, catalog number C7592-100

Limitations
1. Samples with values above 500 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.
2. Patients with severe vitamin B6 deficiency could have a decreased recovery of AST, presumably due to a lack of pyridoxal phosphate.[13]

Calibration
The procedure is standardized by means of the millimolar absorptivity of NADH taken as 6.22 at 340nm under the test conditions described.

Quality Control
The validity of the reaction should be monitored using control sera with known normal and abnormal AST (SGOT) values. These controls should be run at least with every shift in which AST (SGOT) assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Expected Values[13]
8 to 22 IU/L (30°C)
5 to 34 IU/L (37°C)
Since the expected values are affected by age, sex, diet, and geographical location, each laboratory is strongly urged to establish its own reference range for this procedure.

Performance
1. Assay Range: 3-500 IU/L.
2. Comparison: A study was performed between the Yumizen C560 and a similar analyzer using this method, resulting in the following:

Method	AST
N	81
Mean AST (IU/L)	81.9
Range (IU/L)	10-467
Standard Deviation	122.8
Regression Analysis	y = 1.034x + 4.0
Correlation Coefficient	0.9987

3. Precision: Precision studies were performed using the Yumizen C560 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[14]

Within Day

Sample	LOW	MID	HIGH
N	20	20	20
Mean	75.0	268.6	387.2
Standard Deviation	0.8	1.0	1.8
Coefficient of Variation (%)	1.1%	0.4%	0.5%

Total

Sample	LOW	MID	HIGH
N	40	40	40
Mean	71.4	272.2	384.9
Standard Deviation	1.2	2.2	7.3
Coefficient of Variation (%)	1.6%	0.8%	1.9%

4. Sensitivity: 2SD limit of detection (95% Conf) = 3 IU/L

References
1. Tietz, N.W., Fundamentals of Clinical Chemistry, W.B. Saunders co., p 674 (1982).
2. Karmen, A., et al, J. Clin. Invest 34:126 (1955).
3. Henry, R.J., et al, Am. J. Clin. Path. 34:381 (1960).
4. Amador, E., Wacker, W., Clin. Chem. 8:343 (1962).
5. The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology, Scand. J. Clin. Lab. Invest 32:291 (1974).
6. Expert Panel of Enzymes of the International Federation of Clinical Chemistry, Clin. Chem. Acta. 70:F19 (1976).
7. Expert Panel of Enzymes of the International Federation of Clinical Chemistry, Clin. Chem. 24:720 (1978).
8. Jung, K., Bohm, M., Enzyme 23:201 (1978).
9. Hafkenscheid, J.C.M., Dijit, C.C.M., Clin. Chem. 25/1:55 (1979).
10. Horder, M., Bowers, G.N., Jr., Clin. Chem. 23:551 (1977).
11. Henry, R.J., Clinical Chemistry: Principles and Technics, 2nd Ed., Hagerstown (MD), Harper & Row, P882 (1974).
12. Young, D.S., et al, Clin. Chem. 21:1D (1975).
13. Kaplan, L.A., Pesce, A.J., Clinical Chemistry, St. Louis, C.V. Mosby, p.911-912 (1989).
14. NCCLS document “Evaluation of Precision Performance of Clinical Chemistry Devices”, 2nd Ed. (1992).

POINTE

Pointe
AST Reagent Set

HORIBA Instruments Incorporated
5449 Research Drive, Canton, MI 48188
Phone: 734-487-8300; (800) 445-9853
HORIBA Medical

CHEMISTRY PARAMETERS

Chem:	AST	No.:	203	Sample Type:	Serum
Chemistry:	Aspartate Transaminase			Print Name:	AST
Reaction Type:	Kinetic			Reaction Direction:	Negative
Pri Wave:	340			Sec Wave:	412
Unit:	U/L			Decimal	0
Blank Time:	0	0		Reaction Time:	56 71

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard:	6.0 ul	--- ul	--- ul	R1: 120 ul	--- ul
Decreased:	--- ul	--- ul	--- ul	R2: 30 ul	-- ul
Increased:	--- ul	--- ul	--- ul	R3: --- ul	-- ul
	☐ Sample Blank	☑ Auto Rerun		R4: --- ul	--- ul

Slope/Offset Adjustment

Slope: 1 Offset: 0

Linearity Range (Standard)	3	500	Linearity Limit:	0.3
Linearity Range (Decreased)	____	____	Substrate Depletion:	5000
Linearity Range (Increased)	____	____	Mixed Blank Abs:	
R1 Blank Abs:	____	____	Uncapping Time	
Blank Response:	____	____	Reagent Alarm Limit:	
Twin Chemistry:			☐ Enzyme Linear Extension	

☐ Prozone Check ○ Rate Check ○ Antigen Addition

Q1: Q2: Q3: Q4:

PC: ABS:

Pointe
AST Reagent Set

CALIBRATION PARAMETERS

Calibrator Definition

Calibrator: * Lot No.: *

Exp Date: *

Carousel	**Pos**
Sample Carousel 1	*
Sample Carousel 2	
Sample Carousel 3	

Reagent/Calibration

Calibrator	Pos	Lot No	Exp Date	Chem	Conc	Unit
Water	W	*	*	AST	0	U/L

Calibration Setup

Chem: AST

Calibration Settings

Math Model: K Factor

Factor: 4200 Replicates: 1

Acceptance Limits

Cal Time: 24 Hour

Slope Diff: --- SD: ---

Sensitivity : --- Repeatability: ---

Deter Coeff: ---

Auto Calib.

☐ Bottle Changed ☐ Lot Changed ☐ Cal Time

It is recommended that two levels of control material be assayed daily.

* Indicates user defined parameter.

REF 14-A7561-375

Manufactured by
HORIBA Instruments Incorporated-Pointe Brand
5449 Research Drive Canton, MI 48188

2°C 8°C IVD

Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

Manufactured by HORIBA Instruments Incorporated – Pointe Brand
5449 Research Drive, Canton, MI 48188

CE

European Authorized Representative:
Obelis s.a.
Boulevard Général Wahis 53
1030 Brussels, BELGIUM
Tel: (32)2.732.59.54 Fax:(32)2.732.60.03 email: mail@obelis.net

Symbol Key

Use by (YYYY-MM-DD) LOT Lot and batch code REF Catalog number

Manufacturer Temperature limitation Consult instructions for use

IVD *In vitro* diagnostic medical device **Rx Only:** Prescription Use Only