Liquid ALT (SGPT) Reagent Set

Intended Use
For the quantitative determination of Alanine Aminotransferase (ALT) in serum using the Mindray BS-480 analyzer.

Clinical Significance
ALT is widely distributed in tissues with the highest concentrations found in the liver and kidneys. Even so, ALT is considered more liver-specific than AST. Elevated levels of ALT are often only observed in liver diseases such as cirrhosis, hepatitis, or metastatic carcinoma. However, there can be elevated levels of ALT with infectious mononucleosis, muscular dystrophy, and dermatomyositis.[1]

Method History
UV methods for ALT determination were described by Henley[2] in 1955 and Wroblewski and La Due[3] in 1956. The procedure was improved and optimized by Henry et al[4] in 1960. In 1974, the Scandinavian Society for Clinical Chemistry[5] recommended optimized reaction conditions. The International Federation of Clinical Chemistry (IFCC)[6] published a proposed recommended method in 1980 utilizing the LDH-NADH coupled assay. The procedure described herein is based on that method.

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

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{L-Lactate} + \text{NAD}^+ + \text{H}_2\text{O}$$

ALT catalyzes the transfer of the amino group from L-alanine to α-ketoglutarate resulting in the formation of pyruvate and L-glutamate. Lactate dehydrogenase catalyzes the reduction of pyruvate and the simultaneous oxidation of NADH to NAD. The resulting rate of decrease in absorbance is directly proportional to ALT activity.

Reagents
After combining R1 and R2, the reagent contains: L-alanine >450mM, α-ketoglutaric acid >14mM, LDH(microbial) >2000IU/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: Not a hazardous substance or mixture.
Pictogram: Not required.
Signal Word: Not required.
Hazard Statements: Not a hazardous substance or mixture.
Precautionary Statements: Not a hazardous substance or mixture.
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. **Response:** 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. **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-ALT600) available at www.medtestdx.com.**

Specimen Collection and Storage
1. Hemolyzed samples cannot be used as red cells contain ALT.[7]
2. ALT in serum is stable for three days at room temperature (15-30°C), seven days refrigerated (2-8°C), and thirty days frozen (-20°C).[7]

Interferences
1. A number of drugs and substances affect ALT activity. See Young, et al.[8]
2. Bilirubin to at least 30 mg/dl, and hemoglobin to at least 400 mg/dl, have been found to have a negligible effect on this procedure.

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Materials Provided
ALT (SGPT) Reagents R1 and R2

Materials Required but not Provided
1. Mindray BS-480 Analyzer
2. BS-480 Operation manual
3. Chemistry control, catalog number CHEQ480

Limitations
1. Turbid or highly icteric samples may give readings whose initial absorbance exceeds the capabilities of the spectrophotometer. More accurate results may be obtained by using 0.05ml (50ul) of sample and multiplying the final answer by two.
2. Samples with values above 500 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.

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 ALT (SGPT) values. These controls should be run at least with every shift in which ALT (SGPT) 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[9]
4 to 24 IU/L (30°C)
4 to 36 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 Mindray BS-480 and a similar analyzer using this method, resulting in the following:

Method	ALT
N	85
Mean ALT (IU/L)	62.7
Range (IU/L)	8-461
Standard Deviation	95.9
Regression Analysis	y = 1.080x + 2.1
Correlation Coefficient	0.9982

3. Precision: Precision studies were performed using the Mindray BS-480 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[10]

Within Day

Sample	LOW	MID	HIGH
N	20	20	20
Mean	62.4	146.7	349.6
Standard Deviation	1.1	1.2	1.6
Coefficient of Variation (%)	1.7%	0.8%	0.5%

Total

Sample	LOW	MID	HIGH
N	40	40	40
Mean	57.3	142.2	345.9
Standard Deviation	1.2	1.9	4.1
Coefficient of Variation (%)	2.1%	1.3%	1.2%

4. Sensitivity: 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 & 675 (1982).
2. Henley, K.S., Pollard, H.M., J. Lab. Clin. Med. 46:785 (1955).
3. Wroblewski, F., La Due, J.S., Proc. Soc. Exp. Biol. Med. 91:569 (1956).
4. Henry, R.J., et al, Am. J. Clin. Path. 34:381 (1960).
5. The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology, Scand. J. Clin. Lab. Invest 32:291 (1974).
6. Clinica Chimica Acta 105:145F-172F (1980).
7. Henry, R.J., Clinical Chemistry: Principles and Technics, Harper & Row, NY, P522 (1968).
8. Young, D.S., et al, Clin. Chem. 21:1D (1975).
9. Henry, J.B., Clinical Diagnosis & Management by Laboratory Methods, W.B. Saunders Co., Philadelphia, P1437 (1984).
10. NCCLS document “Evaluation of Precision Performance of Clinical Chemistry Devices”, 2nd Ed. (1992).

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CHEMISTRY PARAMETERS

Chem:	ALT	No.:	
Chemistry:	Alanine Transaminase	Print Name:	ALT
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:	0.2
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:

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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	*	*	ALT	0	U/L

Calibration Setup

Chem: ALT

Calibration Settings

Math Model:	K Factor		
Factor:	4700	Replicates:	1

Acceptance Limits

Cal Time:	*	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 ALT480

Manufactured for MedTest DX
5449 Research Drive Canton, MI 48188

2°C 8°C

IVD

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	