

ALT Reagent

(IFCC)

Catalog #: 43703

for use with the

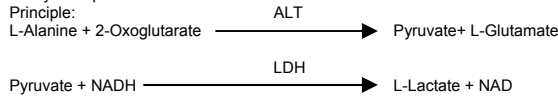
SDI CA480 Clinical Chemistry System

INTENDED USE

For the *in vitro* quantitative determination of Alanine Aminotransferase (ALT) in serum.

METHODOLOGY

UV methods for ALT determination were described by Henley² in 1955 and Wroblewski and La Due³ in 1956. The procedure was improved and optimized by Henry et al⁴ in 1960. In 1974 the Scandinavian Society for Clinical Chemistry⁵ 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.



ALT catalyzes the transfer of the amino group from L-alanine to 2-oxoglutarate resulting in the formation of pyruvate and L-glu-tamate. Lactate dehydrogenase catalyzes the reduction of pyru-vate and the simultaneous oxidation of NADH to NAD. The re-sulting rate of decrease in absorbance is directly proportional to ALT activity. Endogenous sample pyruvate is rapidly and completely reduced by Lactate dehydrogenase (LDH) during the initial incubation period so that it does not interfere with the assay.

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

REAGENT COMPOSITION

Active Ingredients	Concentration
2-Oxoglutarate	13 mM
L-Alanine	440 mM
NADH	0.12 mM
LDH (microbial)	>2000 U/L
Tris Buffer	97 mM

pH 7.8 + 0.1 at 20°C

Precautions:

This reagent is for *In vitro* diagnostic use only.

REAGENT PREPARATION

Reagent is supplied ready to use.

REAGENT STORAGE

1. Store the reagent at 2-8°C (refrigerated).
2. The reagent is stable until the expiration date when stored at 2-8°C.

REAGENT DETERIORATION

DO NOT USE REAGENT IF:

1. The initial absorbance at 340nm is below 1.0.
2. The reagent fails to meet stated parameters of performance.

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 or (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 15 mg/dL, and hemoglobin to at least 500 mg/dL, have been found to have a negligible effect on this procedure.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

1. An SDI CA480 Clinical Chemistry System.
2. Deionized water and related equipment, e.g.: pipettes
3. Analyzer specific consumables, e.g.: sample cups
4. Control material such as those provided by SDI Biomed.

ASSAY PROCEDURE

System Parameters	
ALT (Liquid)	
TEMPERATURE:	37°C
WAVELENGTH:	340 nm
ASSAY TYPE:	Rate/Kinetic
DIRECTION:	Decrease
SAMPLE / RGT RATIO:	1 : 10
e.g. Sample Vol.	0.10mL (100mL)
Reagent Vol.	1.0 mL
DELAY/LAG TIME:	30 Sec
READ TIME:	1 to 3 Min

Procedure Notes:

1. Samples with values above 450 U/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.
2. A very low final reading, together with a small absorbance change between readings could indicate a very high ALT level. Dilute and re-assay as above in Procedure Note "1"
3. 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.

Calculation

One Unit (U/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.

For example:

$$\text{ALT (U/L)} = \frac{\text{DAbs./min} \times 1.10 \times 1000}{6.22 \times 0.10 \times 1.0} = \text{DAbs./min} \times 1768$$

Where:

DAbs./min.	= Average absorbance change per minute
1.10	= Total reaction volume (ml)
1000	= Conversion of U/mL to U/L
6.22	= Millimolar absorptivity of NADH
0.10	= Sample Volume (mL)
1.0	= Light path in cm

Example:

If the average absorbance change per minute = 0.12 then $0.12 \times 1768 = 212$ U/L

NOTES:

1. To convert to nkat/L multiply U/L by 16.67.
2. If any of the test parameters are altered, a new factor must be calculated using the above formula.

CALIBRATION

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

QUALITY CONTROL

The integrity of the reaction should be monitored by use of a two level control with known ALT values.

EXPECTED VALUES⁹

Up to 36 U/L at 37°C

It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE

Linearity:

When run as recommended the assay is linear to 450 U/L.

Method Comparison:

Studies performed between this procedure and a similar procedure yielded the following results:

Correlation Coefficient:	0.999
Slope:	0.95
Intercept:	0.00 (U/L)

Precision:

Within Run	Level 1	Level 2
Mean (U/L)	35	121
S.D. (U/L)	0.7	0.8
C.V. (%)	2.0	0.7

Run to Run

Mean (U/L)	35	121
S.D. (U/L)	0.8	1.2
C.V. (%)	2.3	1.0

Sensitivity:

The sensitivity for this reagent when run as recommended is 0.573 DmA / min per U/L.

REFERENCES

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Manufactured for:



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