ALT

(LIQUID)

3 x 60, 3 x 15 ml

RE – ORDER ALT1020

INTENDED USE

This reagent is intended for the quantitative determination of alanine aminotransferase (ALT) in serum.

METHOD AND HISTORY

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

TEST PRINCIPLE

The ALT catalyzes the conversion of L-alanine and 2-oxoglutarate to pyruvate and L-glutamate. Then LDH catalyzes the oxidation of NADH to NAD.

$$\text{2-oxoglutarate + L-alanine} \xrightarrow{\text{ALT}} \text{L-glutamate + Pyruvate}$$

$$\text{Pyruvate + NADH + H}^+ \xrightarrow{\text{LDH}} \text{Lactate + NAD}^+ \text{ + H2O}$$

ALT catalyzes the transfer of the amino group from L-alanine to *a*-ketoglutarate resulting in the formation of pyruvate and Ll-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.

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.

SPECIMEN COLLECTION

Fresh, clear, unhemolyzed serum is the preferred specimen. No interference is experienced with plasma from commonly used anticoagulants.

Use a standard venipuncture tube to draw patient sample.

The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-200 µl.

Record the patient's name, date and time of sample collection and preparation.

SPECIMEN STORAGE

Specimens for analysis should be stored at 2 to 8°C (refrigerated) and are stable for up to 7 days. Specimens may be stored at -20 to 0°C (frozen) for longer periods. It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur immediately, store the sample properly using the guidelines above.

Reagents necessary for the determination of ALT are included in the kit.

REAGENT

ALT reagent contains, after reconstitution with deionized water:

NADH 1.27 mM	
lactate dehydrogenase (lactobacillus leichmannii)	2800 U/L
alpha ketoglutaric acid	17.5 mM
dL-alanine	583 mM
sodium azide	0.01%
buffer, preservative	

WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact. This reagent contains sodium azide (0.01%) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large amounts of water.

REAGENT PREPARATION

The working reagent is prepared by combining 4 parts of R1 (substrate/enzyme) to 1 part of R2 (coenzyme).

REAGENT STORAGE AND STABILITY

Unopened reagents included in the kits are stable at 2-8° C (refrigerated) until the expiration date stated on the labels. The working reagent is stable at 2-8° C (refrigerated) for 14 days

The initial absorbance of the working reagent read against distilled water at 340 nm (1 cm pathlength) should be at least 0.8 to be considered suitable for use.

ADDITIONAL MATERIALS REQUIRED

Spectrophotometer capable of reading absorbance at 340 nm.

1 cm cuvettes or a flow cell capable of transmitting light at 340 nm.

Test tubes and pipettes.

Timer with one minute increments.

Constant temperature source which can be adjusted to 37°C.

Normal and abnormal control for quality control.

TEST PROCEDURE

The following is a general procedure for use on a manual instrument. Application procedures for use on an automated analyzers are available. Contact King's Technical Service Department for specific information.

PROCEDURE CONDITIONS

Wavelength	340 nm
Temperature	37°C
Pathlength	1.0 cm
Mode	Kinetic
Lag time	1 min
Sample to reagent ratio	1:10

INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 340 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

CALIBRATION

No reagent calibration is necessary as this procedure is standardized based on the millimolar absorptivity of NADH which is taken as 6.22 at 340 nm under the test conditions described.

PROCEDURE

Prepare the required volume of ALT working reagent.

Into separate test tubes pipette 100 µl of serum to be assayed.

Add 1.0 ml of working reagent, mix, and incubate for one to 1 minute at 37°C.

Record the decrease in absorbance at 340 nm at one minute intervals until the absorbance change is constant.

CALCULATION AND RESULTS

$$\text{ALT (U/L)} = \frac{\Delta A/\text{min} \times \text{assay volume (ml)} \times 1000}{6.22 \times \text{light path (cm)} \times \text{sample volume (ml)}} = \Delta A/\text{min} \times 1768$$

ΔA/min = change in absorbance per minute

Assay volume = total reaction volume expressed in ml

1000 = converts U/ml to U/L

6.22 = absorbance coefficient of NADH at 340 nm

Light path = length of the light path expressed in cm (usually 1)

Sample volume = sample volume expressed in ml

1768 = factor derived from constants in the equation

Example: ALT (U/L) =

$$\frac{.017 \times 1.1 \times 1000}{6.22 \times 1 \times 0.1} = .017 \times 1768 = 30 \text{ U/L}$$

0.017 = change in absorbance per minute

1.1 = total reaction volume expressed in ml

1.0 = length of the light path expressed in cm

0.1 = sample volume expressed in ml

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EXPECTED VALUES

The range of expected values is:

4-24 U/L (30° C)

4-36 U/L (37° C)

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

MEDICAL ALERT VALUES

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

LIMITATIONS OF PROCEDURE

This procedure measures total ALT. Red blood cells contain high concentrations of ALT, therefore, hemolysis can elevate results. A summary of the influence of drugs on clinical laboratory test may be found by consulting Young, D.S., et. al.

QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance.

A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

CALIBRATION PROCEDURES

No reagent calibration is necessary as this procedure is standardized based on the millimolar absorptivity of NADH which is taken as 6.22 at 340 nm under the test conditions described.

PRECISION

The estimates of precision shown below were obtained from assays of human control serum.

Within-Run

Mean (U/L)	SD (U/L)	CV (%)
30	0.7	2.3
116	0.8	0.7
381	3.1	0.8

Between-Run

Mean (U/L)	SD (U/L)	CV (%)
31	0.7	2.3
118	1.7	1.4
382	3.5	0.9

CORRELATION

A correlation study was done comparing this method (y) a similar UV alanine aminotransferase procedure (x). Samples range from 7 to 625 U/L. Linear regression analysis gave the following result.

Number of Samples	Regression Equation y=King, x=Comparative	Correlation Coefficient
128	y = 0.96 x + 3.2	.999

LINEARITY

This procedure is linear to 500 U/L A sample with an alanine aminotransferase activity exceeding the linearity limit should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the result.

SENSITIVITY

An absorbance change of 0.0004 ΔA/min corresponds to approximately 1 U/L ALT activity.

REFERENCES

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