Bioway Chemistry Reagent Series

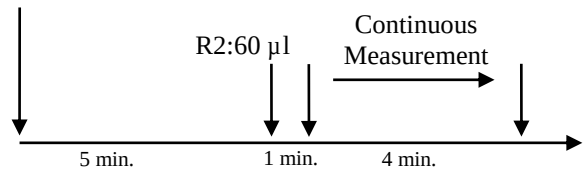
The Serum Alanine aminotransferase Reagent Kit

Detection of Alanine aminotransferase in Human Serum or Plasma on Chemistry Analyzers

Cat. No. R006K11 ***The Serum ALT Reagent Kit***

SUMMARY OF TEST PROCEDURE

Figure 1: Procedure Diagram



*Refer to Figure 1 and the package insert for detail

Table 1: Instrument Parameters*

F factor	3376	Slope of reaction	Decrease
Testing wavelength	Dλ : 340 nm Sλ : 415 nm	Sample volume	15 µl
Test method	Rate Method	R1 volume	240 µl
Reaction temperature	37℃	R2 volume	50 µl

INTENDED USE

Bioway Chemistry Reagent Series **ALT Reagent Kit** (the Kit) is an assay intended for *in vitro* quantitative detection of Alanine aminotransferase in human serum or plasma on automated clinical chemistry analyzers.

SUMMARY AND EXPLANATION

Alanine aminotransferase is also called serum glutamic-pyruvic transaminase ot alanine transaminase. As the name implies, it is responsible for catalyzing the transfer of an amino acid group from L-alanine to α-ketoglutarate. It exists in many tissues, but the maximum level is found in the liver and kidney. ALT is usually used to screen liver problems. Elevated ALT may reflec the possibility of viral hepatitis, cirrhosis and heart diseases.

TEST PRINCIPLES

The Kit utilizes enzymatic and kinetic reactions to measure the ALT activity (U/L) in human serum or plasma.

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

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

A amino group is transferred from alanine to α-ketoglutarate by the catalysis of alanine aminotransferase (ALT) producing Pyruvate. The pyruvate then is reduced to lactate with lactate dehydrogenase (LDH) and oxidase reduced NADH to NAD^+. The process is quantified by measuring the absorabances at 340 nm in a kinetic fashion.

The rate of increase in absorbance at 340 nm is directly proportional to the ALT activity in the sample.

MATERIALS PROVIDED

Reagents:

R1	Tris buffer, pH7.15 NADH L-alanine Lactate dehydrogenase	60 mmol/L 0.18 mmol/L 500 mmol/L 600 U/L
R2	α-Ketoglutarate L-alanine Sodium azide	15 mmol/L 500 mmol/L 1 g/L

MATERIALS NEEDED BUT NOT PROVIDED

1. Automated chemistry analyzer
2. ALT control set (commercially available)

INSTRUMENT

The Kit is applicable on most automated chemistry analyzers. Refer to specific instrument application for suggested settings.

STORAGE AND STABILITY

Store the reagents at 2-8℃. Avoid direct sunlight. The Kit is stable through the expiration date when stored properly. The reagent is stable for 1 month at 2-8℃ after opening.

PRECAUTIONS

1. The Kit is for *in vitro* diagnostic use only. Not for use in humans or animals.
2. The instructions must be followed to obtain accurate results.
3. Do not use the reagents beyond the expiration date.
4. Treat all specimens as infectious. Proper handling and disposal procedures of specimens and test materials should be strictly followed.
5. Reagents contain sodium azide as preservative; avoid contact with skin and eyes, flush with copious amounts of water when disposing.

SPECIMEN COLLECTION AND HANDLING

Follow standard laboratory procedures to collect serum preventing hemolysis.

It is recommended to perform test immediately after sample collection. If the test cannot be done immediately, specimens may be stored at 2~8℃ for 3 days.

TEST PROCEDURE (see Figure 1)

Reagent 1 and 2 are liquid stable ready-to-use, no preparation needed.

Calibration: Recommend using commercially available calibrator set for optimal results.

Test procedure: see Figure 1 and Table 1 for instrument parameter setup. Refer to specific instrument application for suggested setting.

1. Add 15 µl of sample and 240 µl of R1; mix well and incubate at 37℃ for 5 minutes.
2. Add 60µl of R2; mix well and incubate at 37℃ for 1 minute.
3. Take continuous optical density measurement for 4 minutes.
4. Calculate average Δ A /min

RESULT

The ALT activity in U/L can be obtained by the following calculation:

$$\text{ALT (U/L)} = (\Delta A_{test}/\text{min} - \Delta A_{blank}/\text{min}) \times \text{factor (F)}$$

The calculation factor for UV spectrophotometer is 3376 when the optical path is 10 mm. Please refer to instrument application if testing under different conditions.

EXPECTED VALUES

<40U/L
It is recommended for each laboratory to establish its own expected values

QUALITY CONTROL

Using commercially available controls with known concentration is recommended before each batch of tests to ensure the test is properly performed and all reagents and the instrument are functional as specified.

LIMITATIONS

1. The Kit is for *in vitro* use on automated chemistry analyzers only.
2. The test result from the Kit should not be used as the only basis for definite diagnosis.
3. Samples with ALT exceeding the maximum measurement range should be diluted with saline and retested.

PERFORMANCE CHARACTERISTICS

Linearity: 0 - 350 U/L (R≥0.990)

Accuracy: Bias proportion 90%~110%

Precision: Within Run: CV≤4%;
Run-to-Run: CV≤5%

Reagent Blank Absorbance: At 340nm wavelength and 10mmoptical diameter, O.D. ≤0.90.

REFERENCES

1. Kamen A. *et al.*, J. Clin. Inv., 34: 126-133 (1955).
2. Henry R. J. *et al.*, Amer. J. Clin. Path., 34:381 (1960)
3. Wroblewski F. *et al.*, Proc Soc Exp Biol Med, 91:569 (1956)

Not Intended for Sale in the United States.

Pointe Biotech (Nanjing) Co., Ltd.

No.85,Xingmin South Road, Jiang Ning District, Nanjing,P.R.China 211100
Tel:86-25-52425019-800,
Fax:86-25-52425019-866
info@biowaydx.com