ALANINE AMINOTRANSFERASE (ALT)

OSR6107	**4 x 50 mL** **4 x 25 mL**	**R1**
OSR6607*	**4 x 165 mL** **4 x 83 mL**	**R1**

Intended Use
System reagents for the quantitative determination of Alanine Amino-transferase activity (EC 2.6.1.2) in human serum or plasma on Beckman Coulter AU analyzers.
*Alanine Aminotransferase (ALT) reagent OSR6607 for use on the AU2700/5400 system only.

Summary
Alanine aminotransferase (ALT) measurements are useful in the diagnosis and treatment of certain liver diseases (e.g., viral hepatitis and cirrhosis) and heart disease.

ALT is an enzyme involved in amino acid metabolism and is, therefore, found in many tissues. The highest levels of ALT are found in the liver and kidney tissues. Tissue destruction leads to the release of the intracellular enzyme into the circulating blood. Markedly elevated serum ALT levels may be found in a variety of diseases which involve the liver, such as hepatitis, mononucleosis and cirrhosis. These very high levels of ALT are not usually observed in other diseases processes, e.g. myocardial infarction, thus ALT is regarded as a reasonably specific indicator of liver disease.

Methodology
This ALT procedure is based on principles outlined by Wroblewski and LaDue[1] and utilizes a modification of the methodology recommended by the International Federation of Clinical Chemistry (IFCC).[2] ALT transfers the amino group from alanine to α-oxoglutarate to form pyruvate and glutamate. The pyruvate enters a lactate dehydrogenase (LD) catalyzed reaction with NADH to produce lactate and NAD^+. The decrease in absorbance due to the consumption of NADH is measured at 340nm and is proportional to the ALT activity in the sample

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

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

System Information
For AU400/400^e/480, AU600/640/640^e/680 and AU2700/5400 Beckman Coulter Analyzers.

Reagents
Final concentration of reactive ingredients:

Tris buffer, pH 7.15 (37°C)	100	mmol/L
L- Alanine	500	mmol/L
α-Oxoglutarate	12	mmol/L
LDH	≥ 1.8	kU/L
NADH	0.20	mmol/L

Also contains preservative.

Precautions
For *in vitro* diagnostic use.
Do not ingest. Harmful if swallowed.
Contains sodium azide as a preservative which may react with lead joints in copper drain lines to form explosive compounds. Even though the reagent contains minute quantities of sodium azide, drains should be well flushed with water when discarding the reagent.

Preparations of Reagents
For OSR6107, the ALT Reagent is ready for use. No preparation is required. For OSR6607, insert the pipe supplied into the 180mL reagent vial before use on the analyzer. Care must be taken when handling the pipe to avoid contamination. The pipe is for single use only. Do not remove the large cap.

Storage and Stability
1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 - 8°C.
2. Opened reagents are stable for 30 days when stored in the refrigerated compartment of the analyzer.

Indications of Deterioration
Visible signs of microbial growth, turbidity or precipitate, or any change in reagent color may indicate degradation and warrant discontinuance of use.

Specimen Collection And Preparation
Serum or heparinized plasma, free from hemolysis are the recommended samples. EDTA plasma can be used also. However, ALT levels in EDTA plasma should be corrected by multiplying the result obtained by 1.02 to be equivalent to serum levels of ALT. Slight levels of hemolysis will not significantly affect results since erythrocytes contain about 3x - 5x more ALT than serum.[3] Serum should be separated from the red cells as soon as possible after collection.

lanine Aminotransferase (ALT)

Sample Storage and Stability
ALT is stable in serum for 3 days when stored at 2 - 8°C or longer than 3 days when stored frozen at ≤-20°C.[4] ALT is stable in plasma (EDTA) for up to 7 days if stored at room temperature (15 - 25°C) or refrigerated (2 - 8°C).[9]
Cutoff values for blood donor ALT screening should be developed using samples collected and handled in the same way as are routine donor samples.[10]

Interfering Substances
Results of studies[5] show that the following substances interfere with ALT determinations:
The criteria for no significant interference is recovery within 10% of the initial value.

Bilirubin: No significant Interference up to 40 mg/dL Bilirubin
Hemolysis: No significant interference up to 500 mg/dL Hemolysate
Lipemia: No significant Interference up to 300 mg/dL Intralipid*
Pyruvate: No significant Interference up to 1 mmol/L Pyruvate

* Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc., makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young[6] for a compilation of reported interferences with this test.

Procedure
A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

Materials Provided
ALT Reagents
Pipe (one per each 180mL vial)

Stability of Final Reaction Mixture
The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

Calibration
Calibration of this ALT procedure on the AU400/400[e] and AU600/640/640[e] is based upon the theoretical extinction coefficient of NADH, which has a molar absorptivity of 4960 at 340/380 nm. On the AU2700/5400/680/480 it is based on experimental determination of the molar absorptivity at 340/660nm.

Quality Control
During operation of the Beckman Coulter AU analyzer at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Results
Automatically printed out for each sample in U/L at 37°C.

Dynamic Range
The ALT procedure is linear from 3 to 500 U/L. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

Expected Values
Adults[7]: 7 - 52 U/L
Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics
The following data was obtained using the ALT Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision[11]
Estimates of precision, based on CLSI recommendations,[8] are consistent with typical performance. The within run precision is less than 5% CV and total precision is less than 10% CV. Assays of control sera were carried out and data reduced following CLSI guidelines above.

N = 60	Within run		Total	
Mean, U/L	SD	CV%	SD	CV%
24	0.8	3.4	0.9	3.8
144	1.2	0.8	1.7	1.2

Method Comparison[11]
Patient samples were used to compare this ALT Reagent. The table below demonstrates representative performance on the AU analyzers.

Y Method	AU640
X Method	AU600
Slope	1.032
Intercept	0.7
Correlation Coeff. (r)	0.9998
No. of Samples (n)	175
Range (U/L)	3-386

Sensitivity
Typical change in absorbance per minute for 1 U/L of ALT is 0.19 mAbsorbance at 340/380nm and 0.22 mAbsorbance at 340/660nm.

References

1. Wroblewski, F. and LaDue, J.S., Proc Soc Exp Biol Med, 91: 569, 1956.
2. Bergmeyer, H.U. and Horden, M., J. Clin Chem Clin Biochem 18: 521-524, 1980.
3. Murray, R.L.: Alanine aminotransferase In: Methods in Clinical Chemistry, Pesce, A.J., and Kaplan, L.A. eds., C.V. Mosby, St. Louis, 1987.
4. Tietz, N.W. (ed), Textbook of Clinical Chemistry, 2nd Edition, W.B. Saunders, 1994.
5. CLSI/NCCLS, Interference Testing in Clinical Chemistry, EP7-P, 1986.
6. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 5th Edition, AACC Press, 2000.
7. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.
8. CLSI/NCCLS, Evaluation Protocol EP5-T2, 1992.
9. Data on file at Beckman Coulter Inc.
10. Williams, K.M. et al, Transfusion, 27: 431 - 433, 1987.
11. Data is on file for specific AU analyzers.

Manufactured by: Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA

CE