

**Instructions For Use**

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**REF**OSR6007 4 x 12 mL R1, 4 x 6 mL R2  
OSR6107 4 x 50 mL R1, 4 x 25 mL R2  
OSR6607 4 x 173 mL R1, 4 x 91 mL R2  
OSR60180 4 x 17 mL Liquid P-5-P R1-2  
60106 6 x 4 mL Liquid Pyridoxal Phosphate*For in vitro diagnostic use only.***ANNUAL REVIEW**

Reviewed by	Date	Reviewed by	Date

**PRINCIPLE****INTENDED USE**

Kinetic UV test for the quantitative determination of alanine aminotransferase, EC 2.6.1.2 (ALT), in human serum and plasma on Beckman Coulter analysers.

OSR6607 for use on the AU5800, AU2700 and AU5400 systems only.

OSR60180 for use with 3-part-reagent enabled systems only.

**SUMMARY AND EXPLANATION**

Reference<sup>1, 2, 3</sup>

ALT is an aminotransferase, a group of enzymes which catalyse the reversible transformation of  $\alpha$ -keto acids into amino acids by transfer of amino groups. Since the specific activity of ALT in the liver is approximately 10 times that of heart and skeletal muscle, elevated serum ALT activity is mainly regarded as an indicator of parenchymal liver disease. ALT is present in the cytosol of hepatocytes, and increased serum levels indicate deterioration in the integrity of the hepatocyte plasma membrane. ALT has greater diagnostic sensitivity for hepatobiliary disease than AST. Activities >50 times the upper reference limit are mainly associated with acute viral hepatitis, acute disorders of liver perfusion and acute liver necrosis due to ingestion of toxins including paracetamol and carbon tetrachloride. Markedly elevated serum ALT levels may be found in a variety of diseases involving the liver, including hepatitis, mononucleosis and cirrhosis. Elevated ALT levels may be detected in viral hepatitis and other forms of liver disease prior to development of overt clinical symptoms such as jaundice. Levels greater than 15 times the upper reference limit are always indicative of acute hepatocellular necrosis of viral, toxic or circulatory origin. Increased ALT levels may also be detected in cirrhosis and extrahepatic cholestasis. Slight or moderate increases in ALT levels may also be observed after ingestion of alcohol, or administration of drugs including penicillin, salicylates or opiates.

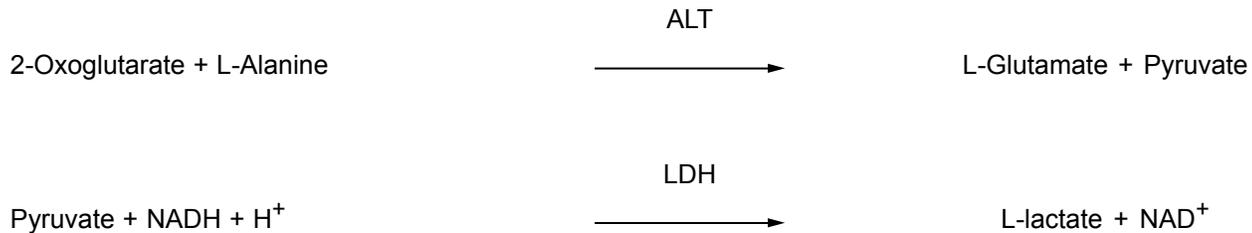
**METHODOLOGY**

Reference<sup>4</sup>

Method based on the recommendations of the “International Federation for Clinical Chemistry” (IFCC).

ALT transfers the amino group from alanine to 2-oxoglutarate to form pyruvate and glutamate. The addition of pyridoxal phosphate to the reaction mixture ensures maximum catalytic activity of ALT. The pyruvate enters a lactate dehydrogenase (LDH) catalysed reaction with NADH to produce lactate and NAD<sup>+</sup>. The decrease in absorbance due to the consumption of NADH is measured at 340 nm and is proportional to the ALT activity in the sample. Endogenous pyruvate is removed during the incubation period.

## CHEMICAL REACTION SCHEME



## SPECIMEN

### TYPE OF SPECIMEN

Serum, and EDTA or heparinised plasma.

Stable in serum and plasma for 7 days when stored at 2...8°C and 3 days when stored at 15...25°C.<sup>5</sup>

Specimen storage and stability information provides guidance to the laboratory. Based on specific needs, each laboratory may establish alternative storage and stability information according to good laboratory practice or from alternative reference documentation.

## REAGENTS

### WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents.

Dispose of all waste material in accordance with local guidelines.

This product contains material of animal origin. The product should be considered as potentially capable of transmitting infectious diseases.

### REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

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

Pyridoxal Phosphate (P-5-P)  
Preservative

0.1 mmol/L (when Cat. No. 60106 or OSR60180 is used)

The concentrations of the reactive components of the reagents shown on the kit label are the actual concentrations in the individual R1/R2 vials. The reagent composition which is shown in the Instructions For Use is the final concentration of these components in the reaction cuvette after addition of R1, Sample, and R2.

 **CAUTION**

**Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76). To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.**

### GHS HAZARD CLASSIFICATION

ALT R1

WARNING

H316

Causes mild skin irritation.

P332+P313

If skin irritation occurs: Get medical advice/attention.

Tris(hydroxymethyl)- aminomethane 1 - 5%

 SDS

Safety Data Sheet is available at [techdocs.beckmancoulter.com](http://techdocs.beckmancoulter.com)

### REAGENT PREPARATION

#### With manual pyridoxal phosphate addition

Pyridoxal Phosphate Liquid (Cat. No. 60106) is supplied for pyridoxal phosphate activation. Pipette Pyridoxal Phosphate Liquid 60106 into the R1 bottle according to the table below, and mix by gentle inversion. R2 is ready for use and can be placed directly on board the instrument. This method can also be used for 3-part-reagent enabled systems.

Cat. No.	Volume Pyridoxal Phosphate Liquid
OSR6007	0.25 mL
OSR6107	1 mL
OSR6607	0.3mL inside the pipe and 3.1mL outside the pipe

Outside the pipe is the gap between the pipe and bottle neck.

#### Without pyridoxal phosphate activation

The reagents are ready for use and can be placed directly on board the instrument.

### STORAGE AND STABILITY

Reagents are stable, unopened, up to the stated expiry date when stored at 2...8°C.

#### Without pyridoxal phosphate activation

Once open, reagents stored on board the instrument are stable for 30 days.

### **With pyridoxal phosphate activation**

After addition of pyridoxal phosphate, R1 stored on board the instrument is stable for 7 days.

R2 stored on board the instrument is stable for 30 days.

### **Pyridoxal Phosphate Liquid reagent (Cat. No 60106)**

Once open, Pyridoxal Phosphate Liquid reagent is stable until the expiry date printed on the label, provided that contamination is avoided through adherence to GLP, the cap is replaced immediately after use and the reagent is stored at 2...8°C.

### **3-part-reagent enabled systems with P-5-P activation**

#### **Reagent Preparation**

P-5-P Liquid (Cat. No. OSR60180) is specifically for use on board 3-part-reagent enabled systems. The reagent is ready for use and can be placed directly on board the instrument in the R1 carousel. R1 and R2 are also ready for use and can be placed directly on board the instrument.

#### **Storage and stability**

Once open, P-5-P (Cat no OSR60180) is stable on board the instrument for 60 days. R1 and R2 reagent stored on board the instrument are stable for 30 days.

## **CALIBRATION**

### **CALIBRATION INFORMATION**

The test is run in MB-mode. To provide a robust approach to generate the analyser specific MB factor it is recommended that 5 separate calibration events should be used. A fresh vial of calibrator, utilising System Calibrator Cat No. 66300 in the AB calibration mode, should be used for each of these runs. When calculating the mean factor from the separate runs the data should be examined for obvious outliers which should be repeated and replaced. For the AU2700/AU5400 this procedure needs to be performed for each ring. Quality control procedures should be undertaken immediately following calibration in accordance with good laboratory practice.

With pyridoxal phosphate activation, the calibrator value is traceable to the IFCC reference method and IRMM/IFCC-454. Without pyridoxal phosphate activation, the calibrator is traceable to a Beckman Coulter Master Calibrator.

Re-establishment of the analyser specific MB factor is recommended when a critical part of the analyser is replaced.

Reagent blank measurement is recommended when changing to a new lot of reagent.

## **QUALITY CONTROL**

Controls Cat. No. ODC0003 and ODC0004 or other control materials with values determined by this Beckman Coulter system may be used.

Each laboratory should establish its own control frequency however good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration/blanking is performed.

The results obtained by any individual laboratory may vary from the given mean value. It is therefore recommended that each laboratory generates analyte specific control target values and intervals based on multiple runs according to their requirements. These target values should fall within the corresponding acceptable ranges given in the relevant product literature.

If any trends or sudden shifts in values are detected, review all operating parameters.

Each laboratory should establish guidelines for corrective action to be taken if controls do not recover within the specified limits.

## TESTING PROCEDURE(S)

Refer to the appropriate Beckman Coulter AU analyser User Guide/Instructions For Use (IFU) for analyser-specific assay instructions for the sample type as listed in the Intended Use statement. The paediatric application is suitable for use with small volume serum/plasma samples.

## CALCULATIONS

The Beckman Coulter analysers automatically compute the ALT activity of each sample.

## REPORTING RESULTS

### REFERENCE INTERVALS

Reference<sup>6, 7</sup>

Male (Adult)	< 50 U/L (0.85 µkat/L)
Female (Adult)	< 35 U/L (0.60 µkat/L)
Newborn/Infant	13 – 45 U/L (0.22 – 0.75 µkat/L)

Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

## PROCEDURAL NOTES

### LIMITATIONS

Highly lipemic samples may exceed the reaction absorbance and will be flagged with a “@”. Such samples should be diluted and re-run.

### INTERFERENCES

Results of studies conducted to evaluate the susceptibility of the method to interference were as follows:

Icterus:	Interference less than 5% up to 40 mg/dL or 684 µmol/L bilirubin
Haemolysis:	Interference less than 10% up to 5 g/L haemoglobin
Lipemia:	Interference less than 3% up to 300 mg/dL Intralipid
Pyruvate:	Interference less than 5% up to 1 mmol/L pyruvate

Patients treated with Sulfasalazine may generate a false low result for ALT.

Refer to Young<sup>8</sup> for further information on interfering substances.

## PERFORMANCE CHARACTERISTICS

### PERFORMANCE CHARACTERISTICS

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

## LINEARITY

The test is linear within an enzyme activity range of 3 – 500 U/L (0.05 – 8.33  $\mu$ kat/L).

## SENSITIVITY

The lowest detectable level in serum on an AU5800 analyser was estimated at 2 U/L.

The lowest detectable level represents the lowest measurable level of ALT that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.

## METHODS COMPARISON

Patient serum samples were used to compare ALT OSR6107 assay on the AU640 against the IFCC reference method. Results of linear regression analysis were as follows:

$y = 1.010x - 0.1$	$r = 0.999$	$n = 117$	Sample range = 3 – 264 U/L
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## PRECISION

The following data was obtained on an AU5800 using 3 serum pools analysed over 20 days.

n = 80 Mean U/L	Within-run		Total	
	SD	CV%	SD	CV%
19	0.38	2.1	0.50	2.7
55	0.68	1.2	1.17	2.1
438	1.70	0.4	4.27	1.0

## ADDITIONAL INFORMATION

DxC 700 AU requires that each reagent application has a standard format of abbreviated Closed Test Name. This Closed Test Name is required to allow automated loading of the calibrator information for each application as part of the DxC 700 AU Closed System. Refer to the table below for the Closed Test Name assigned to each application for this assay.

Test Name	Description
ALT1N	ALT (without P5P)
ALT1NP	ALT (Paediatric without P5P)
ALT2N	ALT (with P5P 60106)
ALT2NP	ALT (Paediatric with P5P 60106)
ALT3N	ALT (with P5P OSR60180)
ALT3NP	ALT (Paediatric with P5P OSR60180)

### Setting Sheet Footnotes

# User defined

\* Values set for working in U/L. To work in SI units ( $\mu$ kat/L) divide by 60.

§ For use in AB mode only, refer to IFU for further instruction.

## **REVISION HISTORY**

IFU updated to add Vietnamese language.

### **Preceding version revision history**

The OSR65XX is now obsolete

Revised Principle

Revised Specimen

Revised Reagent Section

Updated Additional Information section

## REFERENCES

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4. Schumann G, Bonora R, Ceriotti F et al. IFCC Primary Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes at 37°C. Part 4. Reference Procedure for the Measurement of Catalytic Concentration of Alanine Aminotransferase. Clin Chem Lab Med 2002;40:718-24.
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