UDI

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CREATINE KINASE-NAC

(UV/-KINETIC)

REF 028

FOR IN VITRO DIAGNOSTIC USE

INTENDED USE

Quantitative determination of Creatine Kinase (E.C.2.7.3.2) activity in serum/heparinized plasma or EDTA plasma using a UV kinetic method.

DIAGNOSTIC SIGNIFICANCE

Creatine Kinase (CK) plays an important role in the energy storing mechanism of tissue by catalyzing the reversible reaction between creatine and ATP to form creatine phosphate and ADP. CK is distributed in various organs; the highest activities (in decreasing order) are skeletal muscle, heart and brain [1]. Thus determination of CK is an aid to diagnosing muscular dystrophy and other diseases of the skeletal muscles, myocardial infarction, hypothyroidism, renal diseases and/or disfunction[2].

RANGE OF EXPECTED VALUES IN SERUM

ASSAY TEMP	25°C	30°C	37°C
Men	10-80 U/L	15-125 U/L	24-190 U/L
Women	10-70 U/L	15-110 U/L	24-170 U/L

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

METHOD PRINCIPLE

The earlier procedure for determining CK was based on the rate of ATP formation [3]. A modified method was described by Nielson by adding a sulfhydryl compound and AMP to assure maximum CK activity and inhibit adenylate kinase activity [4]. Optimized conditions for measuring CK were published by Szasz in 1976 as well as by the Scandinavian committee on enzymes[5,6]. The above procedure was modified again in 1979 to include EDTA [7]. The UDI CK-NAC reagent is a modification of the above revision

REACTION SEQUENCE:

Creatine Phosphate + ADP $\xleftrightarrow{CK}$ Creatine + ATP

ATP + D-Glucose $\xleftrightarrow{HK}$ Glucose-6-Phosphate + ADP

Glucose-6-Phosphate +NADP $\xleftrightarrow{G\text{-}6\text{-}PDH}$ 6-Phosphogluconate +NADPH+ H^+

REAGENTS

1. CK BUFFER: 100 mmol/L Imidazole buffer (pH 6.7 ± 0.05 at 25 °C; also contains EDTA 2 mmol/L, Magnesium acetate tetrahydrate 20 mmol and Stabilizers. Must be kept tightly capped and protected from contamination.

2. CK-ZYME REAGENT : (concentration based upon reconstitution) 30 mmol/L Creatine phosphate, 20 mmol/L D-Glucose, 20 mmol/L N-Acetyl-L-Cysteine, 2 mmol/L Adenosine diphosphate, 2 mmol/L Adenosine monophosphate, 2 mmol/L NAD, ≥ 2000 U/L Hexokinase from yeast, ≥ 1000 U/L Glucose 6-phosphate dehydrogenase from L. mesenteroides and also contains Filler and Stabilizer. Keep tightly capped and protected from contamination.

RECONSTITUTION

Reconstitute with the amount of CK buffer specified on the individual bottle label. Stable for approximately 7 days after reconstitution when stored at 2 °C to 8 °C or for approximately one month when stored at -20 °C. However, this reagent should not be repeatedly frozen (-20 °C) and thawed.

STORAGE AND STABILITY

Store all reagents supplied with this procedure at 2 °C to 8 °C for maximum stability. All reagents are stable up to expiration date indicated on the individual bottle label.

CHEMICAL PRECAUTIONS

Exercise the normal precautions required for the handling of all laboratory reagents. Pipetting by mouth is not recommended for any laboratory reagent.

INDICATIONS OF REAGENT DETERIORATION

1. Physical Appearance

If CK-ZYME REAGENT appears damp and clumped, deterioration may have occurred and the product should be discarded.

2. Blank Absorbance

If the reconstituted CK REAGENT without sample has an absorbance greater than 0.70 at 340 nm versus reagent grade water, the reagent is considered to be unsatisfactory for use and should be discarded.

3. Control Assays

Failure to obtain accurate results in the assay of control materials may indicate reagent deterioration.

NOTE: UDI cannot guarantee the stability of reagents which have been:

a. transferred from their original containers.
b. improperly stored prior to or during use.
c. contaminated during use.

SPECIMEN

SERUM OR HEPARINIZED PLASMA OR EDTA PLASMA

Collect whole blood by non-traumatic venipuncture and allow to clot. Centrifuge and remove serum immediately. Serum is reportedly[7] stable for 4 hours at room temperature, 8-12 hours at 2 °C to 8 °C, and 2-3 days when frozen(-20 °C) [8].

Hemolyzed specimens should not be used because of side reactions that may occur due to adenylate kinase, adenosine triphosphate, and glucose 6-phosphate dehydrogenase liberated from red cells.

MATERIALS PROVIDED

CK BUFFER and CK-ZYME REAGENT

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

Sample and reagent pipettes, test vials or cuvette, timer, thermoregulated flowcell, spectrophotometer, control serum.

PROCEDURE PARAMETERS

Wavelength .....340 nm
Reaction Type .....Delta Kinetics with factor
Units .....U/L
Factor .....6592 (Semimicro Method) & 4984 (Macro Method)
Incubation Time .....120 Seconds
IntervalTime.....60 Seconds
No.ofIntervals 3
Temperature .....25 °C, 30 °C, 37 °C
ReactionSlope .....Increasing

PROCEDURE (AUTOMATED)

Refer the appropriate instrument application manual available from us.

PROCEDURE (MANUAL)

	SEMI MICRO	MICRO
Reconstituted CK Zyme Reagent	1.0 ml	3.0 ml
Pre-warm at 25°C / 30°C / 37 °C, and add:		
Sample	25µl	100µl
Mix and incubate at 25 °C / 30 °C / 37 °C for 2 minutes and record the absorbance of the sample at 340 nm against reagent grade water. Read the absorbance again after exactly 1, 2 and 3 minutes and determine ΔA/min.		

CALCULATION OF RESULTS

SEMIMICRO METHOD: **ΔA/min x 6592** =CK Activity (IU/L)
MACRO METHOD: **ΔA/min x 4984** = CK Activity (IU/L)

NOTE: If rates greater than 0.12 ΔA/minute are observed, make an appropriate serum dilution with 0.9% saline and repeat the assay. Multiply the results by appropriate dilution factor.

UNIT DEFINITION:

1 IU/L of CK activity is that amount of enzyme which transfers 1 µmol of phosphate from creatine phosphate to ADP per minute per litre of sample with concurrent reduction of 1 µmol of NAD under

specified conditions of the reaction

Factor for calculation has been derived as follows:

$$IU/L = \frac{\Delta A/min \times TV \times 1000 \times 1000}{\varepsilon \times d \times SV}$$

where:

ΔA/min = Change in absorbance per minute, TV = Total volume of reaction mixture (ml), "1000" is a Conversion factor to convert "ml-to-liter" and "1000" is a conversion factor to convert "millimoles-to-micromoles", ε = Molar absorptivity of NADH at 340 nm = 6.22×10^3, d = Light path in cm, SV = Sample volume (ml).

EXAMPLE: If the above semimicro procedure is followed and the ΔA/min = 0.035, TV = 1.025 ml, d = 1-cm, SV = 0.025 ml, absorbance measurement taken at 340 nm; then :

$$IU/L = \Delta A/min \times 6592 = \frac{0.035 \times 1.025 \times 1000 \times 1000}{6.22 \times 10^3 \times 1 \times 0.025} = 230.7$$

CONVERSION FACTOR:To convert from IU/L to SI units (nKat/L) multiply the results by 16.67.

PERFORMANCE CHARACTERISTICS

LINEARITY: The procedure is linear to 1500 IU/L. Values exceeding this concentration should be re-run on smaller samples or dilution and the results to be multiplied by the appropriate dilution factor (also see 'Dilution Effect' under PROCEDURE LIMITATIONS).

COMPARISON: Results obtained using UDI reagent on MANUAL METHOD (y) did not show systematic differences when a similar reagent was used on another AUTO ANALYZER (x) , N = 25

Correlation Coefficient	0.9908
Regression Equation	y = 0.9x + 6.6

PRECISION:

	Mean U/L	SD	CV%
Within run	337.4	7.55	2.24
Run to run	125.1	5.93	4.74

SENSITIVITY: Based upon a photometric resolution of ΔA/minute = 0.001 when semimicro assay procedure as specified above is followed, the demonstrated sensitivity is about 6.6 IU/L.

PROCEDURE LIMITATIONS

1. Some inhibitors of CK activity [9]

a. Excessive Mg^{++}, Cl^-, SO_4^{2-}
b. Most heavy metal, i.e. Zn^{++}, Cu^+, Mn^{++}
c. Iodoacetate and other sulfhydryl binding agents
d. Excess ADP, citrate, fluoride, L-thyroxine
e. Excess uric acid

2. Dilution effect [8, 10]

The dilution of a sample often results in a higher than expected value. Therefore, when CK activity exceeds the upper limit of linearity, a smaller sample volume should be used rather than a diluted sample

3. Lower than expected CK values have been reported [11] in samples having high alkaline phosphatase activity.

4. This procedure measures total CK activity irrespective of its tissue or organ of origin.

5. For a comprehensive review of drug and disease effects on CK levels, see reference 12 and 13.

PROCEDURE NOTES

1. If absorbance measurements are taken at 334 nm, the molar absorptivity of NADH = 6.0×10^3. If absorbance measurements are taken at 336 nm the molar absorptivity of NADH = 3.3×10^3.

2. Some control sera and sera frozen for a few days may exhibit a longer 'lag phase' than 2 minutes; in which case, it may become necessary to wait 4-5 minutes prior to monitoring the change in absorbance so as to allow the reaction to become linear.

3. Traumatic muscle injury (i.e. intramuscular injections) as well as vigorous physical exercise, labour and delivery during pregnancy will elevate the CK value.

4. CK is a light sensitive enzyme and excessive light exposure will reportedly cause CK values to decrease in the serum sample.

TEMPERATURE CONVERSION FACTORS

You may convert results to approximate results at other temperatures by multiplying the appropriate temperature factor.

ASSAY TEMPERATURE	REPORTING TEMPERATURE		
	25°C TF	30°C TF	37°C TF
25°C	1.00	1.38	2.3
30°C	0.72	1.00	1.67
37°C	0.43	0.600	1.00

QUALITY CONTROL

For accuracy and precision check, we recommend use of normal and abnormal UDI controls based on human serum.

ORDERING INFORMATION:

UDITROL 'N' (Normal Serum Control) REF # 070N-010 2x5 ml
UDITROL 'A' (Abnormal Serum Control) REF#070A-010 2x5 ml

REFERENCES

1. Faulker, W.R. and Meites, S.: Selected Method of Clinical Chemistry. Vol. 9, p. 185 (1982).
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5. Szasz, G et al, Clin Chem, 22:650 (1976).
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7. The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology, Scand. J Clin Lab Invest, 36:711 (1979).
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12. Young, D.S. et al., Clin. Chem., 21:1D (1975).
13. Friedman, RB et al. Clin Chem, 26:1D (1980).

PRODUCT AVAILABILITY

CREATINE KINASE - NAC (UV/KINETIC)

REF # 028-060	**10 x 6 ml**
REF # 028A-060	**20 x 3 ml**
REF # 028-024	**4 x 6 ml**
REF # 028-012	**2 x 6 ml**

EC REP
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Germany

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LACTATE DEHYDROGENASE (LDH-L) REAGENT UV/KINETIC (MOD. AMADOR WACKER) METHOD

REF 047

FOR IN VITRO DIAGNOSTIC USE

INTENDED USE

Quantitative determination of lactate dehydrogenase, LDH(L-LACTATE: NAD OXIDOREDUCTASE, E.C. 1.1.1.27), in serum or plasma using a lactate to pyruvate kinetic method.

DIAGNOSTIC SIGNIFICANCE

Elevated levels of LDH-L in serum are found in myocardial infarction, liver disease, renal disease, certain forms of anemia, malignant diseases and progressive muscle dystrophy[1,2].

RANGE OF EXPECTED VALUES[3]

ASSAY TEMP.	30 °C	37 °C
MALES	50-166 U/L	80-285 U/L
FEMALES	60-132 U/L	103-227 U/L

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

METHOD PRINCIPLE

LDH catalyzes the following reaction:

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

The rate of NADH formation is indicated by increase in absorbance at 340 nm and is directly proportional to serum LDH-L activity.

The UDI procedure is based upon the modification of Amador Wacker method[4,5]

REAGENTS

1. **LD ZYME REAGENT (concentration based upon reconstitution):70 mmol/l Lithium L-Lactate, NAD 5.5 mM, 80 mmol/L Buffer pH 8.6± 0.05 (25 °C); Non-reactive stabilizers.**

REAGENT STORAGE & STABILITY

Store all reagents in this set at 2-8 °C. All reagents are stable upto expiration date indicated on individual bottle.

CHEMICAL PRECAUTIONS

Exercise the normal precautions required for the handling of all laboratory reagents. Pipetting by mouth is not recommended for any laboratory reagents.

INDICATIONS OF REAGENT DETERIORATION

1. Physical appearance:
   If the dry, powdered reagent does not have a white to off-white appearance, the reagent may have deteriorated. If the reagent blank before serum addition exceeds 0.45 at 340 nm, the reagent may have deteriorated.
2. Control Assays:
   Failure to obtain accurate results in the assay of control material may indicate reagent deterioration.

NOTE: UDI cannot guarantee the stability of reagents which have been:

a) transferred from their original containers
b) improperly stored
c) contaminated during use.

METERIALS PROVIDED

LD Zyme Reagent.

ADDITIONAL MATERIALS REQUIRED, BUT NOT PROVIDED

Sample and reagent pipettes, Test Vials or Cuvettes, Timer,Spectrophotometer with temp. controlled flow cell, Control Sera.

SPECIMEN

SERUM, HEPARINZED PLASMA OR EDTA PLASMA

Hemolysis must be avoided, since red cells contain 150 times more LD than serum. LDH in serum is reported stable for 2-3 days at room temperature [2]

REAGENT PREPARATION

Reconstitute a vial of LD Zyme reagent with the volume of distilled water stated on vial label. Gently swirl to dissolve. Reconstituted reagent is stable for 14 days at 2-8 °C. Do not use if absorbance of blank exceeds 0.450 at 340 nm.

PROCEDURE PARAMETERS:

Wavelength .....340 nm (340/380 Bichromatic Filter)
ReactionType.....Rate
Reaction Direction.....Up
Reaction Temperature.....30 °C or 37 °C
Measurement time.....60-180 seconds
Linearity.....500 U/L
Sample Volume.....25 µL (Semimicro) or 100µL (Macro)

Factor .....6592 (Semimicro) or 4984(Macro)

PROCEDURE (AUTOMATED)

Refer to the appropriate instrument application manual available from us.

PROCEDURE (MANUAL)

This procedure can be performed either by Semimicro method or by Macro method. Pipette into clean cuvette

	SEMIMICRO	MACRO
Reconstituted LD Zyme Reagent	1.0 ml	3.0 ml
Pre-warm at 30 °C or 37 °C for 3 minutes and add:		
Sample	0.025 ml	0.1 ml
Mix and incubate at 30 °C/37 °C for 1 minute, then read absobance (at 340 nm against distilled water) every minute for 3 minutes (with incubation at 30 °C or 37 °C between readings) and determine ΔA /min.		

CALCULATION OF RESULTS:

Multiply ΔA/min. with a factor of 6592 (Semimicro method) or 4984 (macro method) to get results in U/L at that particular temperature.

EXAMPLE: Assume that ΔA/min. of a sample by semimicro method at 37 °C was 0.019, 0.020 over a period of 3 minutes. The average ΔA/min is 0.0195. Therefore, LDH-L activity at 37 °C = ΔA/min x 6592 = 0.0195 x 6592 = 128.5 U/L.

UNIT DEFINITION: One Unit (U/L) is the amount of enzyme that will reduce one micromole of NAD per minute per liter of sample at specified temperature.

The following formula was used to derive the factors for calculating LDH-L activity in U/L.

$$U/L = \frac{\Delta A/\text{min} \times TV \times 10^6}{\varepsilon \times d \times SV}$$

Where

ΔA/min = Measured rate of change in absorbance per minute
TV = Total volume of reaction mixture (ml)

10^6 = Factor to convert millimole/ml to micromole/liter
ε = Molar absorptivity of NADH at 340 nm = 6.22×10^3 L Xmole $^{-1}$ cm^{-1}
d = Light path in cm
SV = Sample Volume (ml)

For Semimicro Method

$$U/L = \frac{\Delta A/min \times 1.025 \times 10^6}{6.22 \times 10^3 \times 1 \times 0.025} = \Delta A/min \times 6592$$

For Macro Method

$$U/L = \frac{\Delta A/min \times 3.1 \times 10^6}{6.22 \times 10^3 \times 1 \times 0.1} = \Delta A/min \times 4984$$

SPECIFIC PERFORMANCE CHARACTERISTICS

1. LINEARITY and SENSITIVITY: LDH values exceeding 500U/L should be re-run on dilution. Typically, 0.001 represents about 5.0 U/L by Macro method and 6.6 U/L by Semimicro method .

2. COMPARISION: UDI reagent tested on MANUAL (y) was compared with CAPS survery results and similar UDI reagent for other systems (x). The systematic difference between the results were within CLIA specified limits, N=36

Correlation Coefficient	0.9901
Regression Equation	y=1.043x - 7.5

3. PRECISION:

	Mean (U/L)	SD	CV%
Within run	250.4	3.80	1.52
Run to run	130.6	5.05	3.87

PROCEDURE NOTES

1. If ΔA/min. is greater than 0.100 at 340 nm , the test should be repeated using a serum diluted 1:10 with physiological saline; multiply the result by 10.
2. The procedure measures total lactate dehydrogenase irrespective of its tissue or organ of origin.
3. For a comprehensive review of drug and disease effect on "*in vitro*" and "*in vivo*" LDH levels, see ref: 6, 7.
4. The reaction temperature must be maintained to within ± 0.1 °C, during the assay.
5. If the relationship between the slope of the instrument and the molar absorptivity of NADH is not known the results may be inaccurate. It is very important that wavelength calibration be performed stray, light be kept to minimum and instrument drift be ascertained.
6. Temperature conversion factor (Tf)[3]

Assay Temp.	Tf at different result reporting temperatures	
	30 °C	37 °C
30 °C	1.00	1.7
37 °C	0.6	1.00

Example: If the assay is performed at 30 °C but is to be reported at 37 °C simply multiply the results by factor 1.7. If the assay is performed at 37 °C but is to be reported at 30 °C simply multiply the results by 0.6.

NOTE: Since temperature factors give only an approximate conversion, it is suggested that values be reported at temperature of measurement.

QUALITY CONTROL

For accuracy and precision check, we recommend the use of normal and abnormal UDI controls based on human serum.

ORDERING INFORMATION

UDITROL 'N' (Normal Serum Control) REF # 070N-010 (2x5 ml)

UDITROL 'A' (Abnormal Serum Control) REF # 070A-010 (2x5 ml)

REFERENCES

1. Henry J.B., Clinical Diagnosis and Management by Laboratory Methods,W.B. Saunders and Company, Philadelphia, PA, P 365 (1979)
2. Tietz, R.W, Fundamentals of Clinical Chemistry, WB Saunders & Company Philadelphia, PA, P 652 (1976).
3. Henry R.J., et al., Clinical Chemistry; Principles and Techniques 2nd ED.,Harper and Row, Hagers Town (MD) PP 819-831 (1974)
4. Amador, E. et al. Clin Chem. 9:391 (1963)
5. Wacker, WEC et al, New Eng. J. Med. 225:449 (1956)
6. Young. DS et al, Clin Chem, 21:1D (1975).
7. Friedman, RB et al, Clin Chem, 26:1D (1980).

PRODUCT AVAILABILITY

LDH-L REAGENT SET (UV/KINETIC)

REF # 047-050	**10 x 5 ml**
REF # 047-060	**20 x 3 ml**
REF # 047-240	**20 x 12 ml**
REF # 047-024	**2 x 12 ml**

EC REP
(Authorized representative in the European Community)

mdi Europa GmbH
Wittekamp 30
D-30163 Hannover
Germany

CE

COD 11580 1 x 50 mL	COD 11581 1 x 200 mL
STORE AT 2-8°C	
Reagents for measurement of LDH concentration Only for *in vitro* use in the clinical laboratory	

LACTATE DEHYDROGENASE (LDH)

BioSystems
REAGENTS & INSTRUMENTS

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LACTATE DEHYDROGENASE (LDH)
PYRUVATE

PRINCIPLE OF THE METHOD

Lactate dehydrogenase (LD or LDH) catalyzes the reduction of pyruvate by NADH, to form lactate and NAD^+. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm[1,2].

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

CONTENTS

	COD 11580	COD 11581
A. Reagent	1 x 40 mL	1 x 160 mL
B. Reagent	1 x 10 mL	1 x 40 mL

COMPOSITION

A. Reagent: Tris 100 mmol/L, pyruvate 2.75 mmol/L, sodium chloride 222 mmol/L, pH 7.2

B. Reagent: NADH 1.55 mmol/L, sodium azide 9.5 g/L.

Harmful: (Xn). R22: Harmful if swallowed. R31: Contact with acids liberates toxic gas. S28.1: After contact with skin, wash immediately with plenty of water.

STORAGE

Store at 2-8°C.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

- Reagent: Presence of particulate material, turbidity, absorbance of the blank lower than 1.200 at 340 nm (1 cm cuvette).

REAGENT PREPARATION

Working Reagent. Pour the contents of the Reagent B into the Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B. Stable for 2 months at 2-8°C.

ADDITIONAL EQUIPMENT

- Analyzer, spectrophotometer or photometer with cell holder thermostatable at 25, 30 or 37°C and able to read at 340 nm.
- Cuvettes with 1 cm light path.

SAMPLES

Serum or plasma collected by standard procedures. Serum or plasma must be separated from the clot as soon as possible. Do not use hemolysed samples.

Lactate dehydrogenase in serum or plasma is stable for 2 days at room temperature and for 24 hours at 2-8°C. Use heparin as anticoagulant.

PROCEDURE

1. Bring the Working Reagent and the instrument to reaction temperature.
2. Pipette into a cuvette: (Note 1)

Working Reagent	1.0 mL
Sample	20 µL

3. Mix and insert the cuvette into the photometer. Start the stopwatch.
4. After 30 seconds, record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
5. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (ΔA/min).

CALCULATIONS

The LDH concentration in the sample is calculated using the following general formula:

$$\Delta A/\text{min} \times \frac{Vt \times 10^6}{\varepsilon \times l \times Vs} = U/L$$

The molar absorbance (ε) of NADH at 340 nm is 6300 and the lightpath (l) is 1 cm, the total reaction volume (Vt) is 1.02, the sample volume (Vs) is 0.02 and 1 U/L are 0.0166 µkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

ΔA/min	x 8095 = U/L x 135 = µkat/L

REFERENCE VALUES

Reaction temperature	Adults U/L	Adults µKat/L
25°C	105-210	1.70-3.50
30°C[2]	140-280	2.30-4.70
37°C[1]	207-414	3.40-6.80

Values at 25°C are obtained from those at 30°C by using a conversion factor.These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 4.7 U/L = 0.078 µkat/L.
- Linearity limit: 1250 U/L = 20.92 µkat/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Repeatibility (within run):

Mean Concentration	CV	n
324 U/L = 5.40 µkat/L	3.9 %	20
1029 U/L = 17.15 µkat/L	2.3 %	20

- Reproducibility (run to run):

Mean Concentration	CV	n
324 U/L = 5.40 µkat/L	6.6%	25
1029 U/L = 17.15 µkat/L	3.3 %	25

- Sensitivity: 0.123 ΔmA·L/U·min = 7.41 ΔmA ·L/µkat·min.
- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.
- Interferences: Hemolysis interferes due to the high lactate dehydrogenase concentration in red cells. Lipemia (triglycerides < 10 g/L) and bilirubin (< 20 mg/dL) do not interfere. Other drugs and substances may interfere[3].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Lactate dehydrogenase is present in all cells of the body but its higher concentrations are found in liver, heart, kidney, skeletal muscle and erythrocytes.

Total LDH concentration in serum or plasma is increased in patients with liver disease, renal disease, myocardial infarction, many malignant diseases, progresive muscular dystrophy and almost any cause of hemolysis[4,5].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. This reagent may be used in several automatic analysers. Instructions for many of them are available on request.

BIBLIOGRAPHY

1. Sociedad Española de Química Clínica, Comité Científico, Comisión de Enzimas. Método recomendado para la determinación en rutina de la concentración catalítica de lactato deshidrogenasa en suero sanguíneo humano. Quim Clin 1989; 8: 57-61.
2. Scientific Commitee. Recommandations pour la mesure de la concentration catalytique de la lactate deshidrogenase dans le serum humain a 30°C. Ann Biol Clin 1982; 40: 87-164.
3. Young DS. Effects of drugs on clinical laboratory tests, 4th ed. AACC Press, 1995.
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5. Friedman and Young. Effects of disease on clinical laboratory tests, 3th ed. AACC Press, 1997.

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United Diagnostics Industry
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ALT (SGPT) UV/ KINETIC

REF 007

FOR IN VITRO DIAGNOSTIC USE ONLY

INTENDED USE

Quantitative determination of glutamate pyruvate transaminase (SGPT; alanine aminotransferase; EC 2.6.1.2) in serum/plasma using a UV rate method.

DIAGNOSTIC SIGNIFICANCE

Alanine aminotransferase (ALT) is found mainly in liver cells: thus, an elevated ALT is a sensitive index of acute heptocellular injury. Elevated serum ALT (SGPT) levels are found in hepatitis, cirrhosis, and obstructive jaundice. Levels of ALT (SGPT) are only slightly elevated in patients following a myocardial infarction [1]

RANGE OF EXPECTED VALUES IN SERUM

Assay Temperature	30 °C	37 °C
Men	up to 30 U/L	up to 42 U/L
Women	up to 23 U/L	up to 32 U/L

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

METHOD PRINCIPLE

ALT catalyzes the transfer of the amino group from L-alanine to 2-oxoglutarate, producing pyruvate and L-glutamate. The pyruvate formed in the reaction is reduced to L-lactate by lactic dehydrogenase (LDH) with the simultaneous oxidation of nicotinamide adenine dinucleotide (NADH). The rate of decrease in absorbance at 340 nm is proportional to the ALT activity of the sample.

L-ALANINE + 2-OXOGLUTRATE $\xleftarrow{ALT}$ PYRUVATE + L-GLUTAMATE

PYRUVATE + NADH + H^+ $\xleftarrow{LDH}$ L-LACTATE + NAD^+ + H_2O

The UDI ALT reagent conforms to the formulation recommended by the IFCC[2] based on the Wroblewski and La Due procedure[3].

REAGENTS

ALT (SGPT) REAGENT : (Concentrations refer to reconstituted reagent) 100 mmol/L Tris, 350 mmol/L L-Alanine, 15 mmol/L 2-Oxoglutarate with Preservative; 0.25 mmol/L NADH, ≥5000 U/L LDH with Filler and Stabilizer, pH 7.5 ± 0.05 (25 °C). Must be kept tightly capped and protected from contamination. Must be kept dry. Can be used until the expiration date indicated on the individual bottle label.

RECONSTITUTION

Reconstitute each vial of ALT (SGPT) Reagent with the volume of distilled water indicated on the vial label. After the addition of distilled water, swirl gently to dissolve. DO NOT AGITATE VIGOROUSLY as this may denature the enzyme in the reagent. Stable for 7 days at 2-8 °C.

STORAGE INSTRUCTIONS

ALT (SGPT) Reagent must be stored under refrigeration (2-8 °C) for maximum stability.

CHEMICAL PRECAUTIONS

Exercise the normal precautions required for the handling of all laboratory reagents. Pipetting by mouth is not recommended for any laboratory reagent.

INSTRUMENT REQUIREMENTS

Any calibrated spectrophotometer which gives a linear response and meets or exceeds the following specifications is satisfactory for use with this procedure:

Wavelength 340nm
Sensitivity 0.001A
Light Path .....10-mm parallel-faced cuvette
Thermo regulated Cuvette ..... 30 °C/37 °C
Band pass ..... 8nm or less

SPECIMEN

Serum or Heparinized Plasma or EDTA Plasma

Collect whole blood by venipuncture and allow to clot. Centrifuge and immediately remove serum. AVOID HEMOLYSIS. ALT activity in serum reportedly[4] is stable for 48 hours at room temperature, seven days at 2-8 °C and at least 8 months at -20 °C.

MATERIAL PROVIDED

ALT (SGPT) REAGENT

ADDITIONAL MATERIALS REQUIRED, BUT NOT PROVIDED.

Reagent and sample pipettes, test vials, timer, spectrophotometer with thermo regulated cuvette, control serum.

PROCEDURE PARAMETERS

Wavelength ..... 340 nm (340/380 Bichromatic Filter)
Reaction Type ..... Delta kinetics with Factor
Units ..... U/L
Factor .....1768 (Semi micro Method)
Incubation Time ..... 60 Seconds
Interval Time ..... 60 Seconds
Number of Intervals 2
Temperature .....30 °C/37 °C
Reaction Slope ..... Decreasing

PROCEDURE (AUTOMATED)

Refer the appropriate instrument application guide available from us.

PROCEDURE (MANUAL)

1. SEMIMICRO METHOD

	TEST
Reconstituted ALT (SGPT) Reagent	1.0 ml
Pre-warm at 30 °C/37 °C and add :	
Sample	0.1 ml
Mix, and aspirate into the thermo regulated flow cell set at 30°C/37°C	
Allow 60 seconds for temperature equilibration. Read the absorbance (at 340 nm against distilled water) every minute for 3 min. to determine ΔA/Min.	

CALCULATION OF RESULTS

ΔA/Min x 1768 = ALT activity (U/L)

EXAMPLE: If the average absorbance change per minute of a sample at 37 °C is 0.02, then its ALT activity at 37 °C would be 0.02 x 1768 = 35.4 U/L.

II. MACRO METHOD.

Pipette into a clean cuvette :

	TEST
Reconstituted Reagent	3.0 ml
Pre-warm at 30 °C/37 °C, then add :	
Sample	0.2 ml
Mix, and incubate at 30°C/37°C for 1 minute and read the absorbance (A_1) at 340 nm against distilled water. Re-incubate at 37 °C and after exactly 3 minutes read the absorbance (A_2).	

CALCULATION OF RESULTS

(A_2 - A_1) x 857 = ALT Activity (U/L)

UNIT DEFINITION: One international unit (U) is the amount of enzyme that will cause the transamination of one micromole of L-alanine per minute under the specified conditions of the reaction.

The following formula is used to derive the factor for calculating ALT activity in U/L.

$$U/L = \frac{\Delta A/min \times TV \times 10^6}{\varepsilon \times SV \times L}$$

where: ΔA/min = Measured rate of change in absorbance per minute
TV = Total volume of reaction mixture (ml)
10^6 = Factor to convert moles to micromoles
ε = Molar absorptivity of NADH at 340 nm (6.22×10^3 L x Mole $^{-1}$ cm^{-1})
SV = Sample volume (ml)
L = Light Path (cm)

FOR SEMIMICRO METHOD

$$\frac{\Delta A/min. \times 1.1 \times 1000}{6.22 \times 0.1 \times 1} = \Delta A/Min. \times 1768 \text{ U/L}$$

FOR MACRO METHOD

$$\frac{A_2 - A_1 \times 3.2 \times 1000}{3 \times 6.22 \times 0.2 \times 1} = (A_2 - A_1) \times 857 \text{ U/L}$$

PROCEDURE LIMITATIONS:

1. If the ΔA/Min. is greater than 0.142 (activity greater than 250 U/L), Repeat the test using less volume of samples.
50µl . . . . . .calculation factor would then be ΔA/Min x 3376
25µl . . . . . .calculation factor would then be ΔA/Min x 6592
2. The presence of fluoride or heavy metals may falsely depress ALT values through enzymatic inhibition[3].
3. Ammonia can react with 2-oxoglutarate if increased levels of glutamate dehydrogenase are present in the sample and lead to the oxidation of NADH which would result in falsely elevated ALT levels. Care must be taken to keep ammonium ions from entering the reaction system.
4. Elevated ALT or α-keto acid levels may significantly reduce the NADH concentration prior to measuring the absorbance during the reaction interval resulting falsely decreased ALT values.
5. For a comprehensive review of the "in vivo" and "in vitro" drug and disease effects on ALT testing, see references 6 and 7.
6. The procedure measures total serum ALT irrespective of its tissue or organ of origin. Additional tests may be necessary for differential diagnosis.

PROCEDURE NOTES

1. A major cause of interlaboratory variability is the failure to calibrate the spectrophotometer, i.e. wavelength peak and absorbance[4].
2. Initial absorbance is a very important indicator of potential assay problems, i.e. initial absorbance less than 0.800 suggests possible substrate depletion whereas initial absorbance greater than 1.600 suggests turbid or icteric sample which requires sample dilution.
3. If "U/L" is to be converted to SI units (nKat/L), use the conversion factor U/L = 16.67 nKat/L
4. The ALT activity is very temperature sensitive. So that if a temperature other than 30°C is used, the effect of the temperature on the measured ALT activity must be measured.
5. Pyridoxal-5'-Phosphate activation has not been included in the procedure. If prior P-5'-P activation is performed, it is important to re-assess the normal values used within your laboratory. It is also important to note that quality control sera will almost invariably reflect higher activities with P-5'-P activation.

TEMPERATURE CONVERSION FACTORS (T_f)[4]

1. If the assay is performed at 37 °C but is to be reported at 30 °C, multiply the results by 0.7.
2. If the assay is performed at 30 °C but is to be reported at 37 °C, multiply the results by 1.43.

PERFORMANCE CHARACTERISTICS

COMPARISON : UDI reagent tested on MANUAL SYSTEM(y) was compared with similar UDI reagent for other systems (x) which in turn is matching with CAPS survey results. The reagent was also compared with another commercial reagent. The systematic difference between the results were within CLIA specified limits, N = 26
Correlation Coefficient 0.99
Regression Equation $y = 0.93x - 1.48$

PRECISION:

	Mean U/L	SD	CV%
Within run	44.4	1.52	3.42
Run to run	33.5	1.90	5.67

QUALITY CONTROL

For accuracy and precision check, we recommend use of normal and abnormal UDI controls based on human serum.

ORDERING INFORMATION:
UDITROL 'N' (Normal Serum Control)
REF # 070N-010 2x5 ml
UDITROL 'A' (Abnormal Serum Control)
REF # 070A-010 2x5 ml

BIBLIOGRAPHY

1. Henry, J.B.: Clinical Diagnosis and Management by Laboratory method. W.B. Saunders Co., Philadelphia, PA. P 332-335 (1974).
2. International Federation of Clinical Chemistry, J. Clin. Chem. Clin Bio. 18:5231 (1980).
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4. Butler, TJ., Selected Methods of Clinical Chemistry, Vol 9. Eds, WR Faulkner and S Meltes, AACC, Washington,DC, pp 69-73 (1982).
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7. Friedman, RB et al, Clin Chem, 26:1D (1975).
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PRODUCT AVAILABILITY

ALT (SGPT) UV/KINETIC

REF # 007-240	**20 x 12 ml**
REF # 007-120	**8 x 15 ml**
REF # 007-100	**2 x 50 ml**
REF # 007-060	**5 x 12 ml**
REF # 007A-060	**20 x 3 ml**
REF # 007-050	**1 x 50 ml**

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AST (SGOT) REAGENT SET
(UV/-KINETIC METHOD)

REF 015

FOR IN VITRO DIAGNOSTIC USE

INTENDED USE

For the quantitative determination of Aspartate Aminotransferase (AST) in serum, by a UV Kinetic method.

DIAGNOSTIC SIGNIFICANCE

Serum aspartate aminotransferase (AST) also known as serum glutamate oxaloacetate transaminase (SGOT) is a tissue enzyme that catalyzes the exchange of amino and keto groups between alpha-amino acids and alpha-keto acids. AST is widely distributed in tissues, principally cardiac, hepatic, muscle and kidney. Injury to these tissues results in the release of the AST (SGOT) enzyme to general circulation. Follwoing a myocardial infarction, serum levels of AST (SGOT) are elevated and reach a peak 48 to 60 hours after onset. Hepatobiliary diseases such as cirrhosis, metastatic carcinoma and viral hepatitis also will increase serum AST levels[1].

RANGE OF EXPECTED VALUES IN SERUM

ASSAY TEMP.	37 °C
MEN	upto 37 U/L
WOMEN	upto 31 U/L

It is strongly recommended that each laboratory should establish its own "normal" values.

METHOD PRINCIPLE

The first kinetic assay of AST for diagnostic purposes was described by Karmen et al. in 1955, using a coupled reaction of malate dehydrogenase (MDH) and NADH[2]. This assay system was critically evaluated and optimized in 1960 by Henry et al[3]. In 1977 the International Federation of Clinical Chemistry recommended a reference procedure for the measurement of AST activity based upon Karmen's procedure[4]. The AST reagent applies the formulation recommended by the IFCC.

The enzymatic reaction sequence employed in the assay of aspartate aminotransferase is as follows:

$$\text{L-Aspartate} + \text{2-Oxoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{L-Glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xrightarrow{\text{MDH}} \text{L-Malate} + NAD^+ + H_2O$$

AST catalyzes the transfer of an amino group between L-aspartate and 2-Oxoglutarate. The oxaloacetate formed in the first reaction is then reacted with NADH in the presence of malate dehydrogenase (MDH) to form NAD. AST activity is determined by measuring the rate of oxidation of NADH at 340 nm. Lactate dehydrogenase is included in the reagent to convert endogenous pyruvate in the sample to lactate during the lag phase prior to measurement.

REAGENT COMPOSITION

AST (SGOT) REAGENT : 2-Oxoglutarate 12 mM, L-Aspartic Acid 200 mM, NADH 0.19 mM, LDH 800 U/L, MDH 600 U/L, Buffer 100 mM, pH 7.8 ± 0.1 and non-reactive Preservatives and Fillers. (Concentrations refer to reconstituted reagent).

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use.

CAUTION: In vitro diagnostic reagents may be hazardous. Handle in accordance with good laboratory procedures which dictate avoiding ingestion and eye or skin contact.

2. Specimens should be considered infectious and handled appropriately.

3. Use distilled or deionized water where indicated.

STORAGE AND STABILITY

1. Store dry reagent at 2-8 °C

2. The reconstituted reagent is stable for eight (8) hours at room temperature (below 30 °C) and for twenty one (21) days when refrigerated (2-8 °C) immediately.

REAGENT DETERIORATION

The reagent should be discarded if:

1. The initial absorbance, read against water at 340 nm, is below 0.800.

2. The reagent fails to meet the stated parameters of performance.

MATERIALS PROVIDED

AST (SGOT) Reagent.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

Reagent and sample pipettes, test vials, rack, Timer, spectrophotometer with capability to read at 340 nm (uv) preferably with a thermoregulated cuvette and / heating bath and control sera.

SPECIMEN

SERUM : Reports indicate that AST in serum remains stable at 2-8 °C for a minimum of seven (7) days. Hemolyzed specimens should not be used as erythrocytes contain fifteen times the AST activity in serum[5].

REAGENT PREPARATION

Reconstitute one vial of AST(SGOT) reagent with the volume of distilled water specified on the label. Mix by gentle inversion. Do not shake vigorously.

PROCEDURE PARAMETERS

Reaction type .....Delta kinetics with factor
Units .....U/L
Factor .....1768 (Semi micro method)
Incubation time .....60 Seconds
Interval time .....60 Seconds
No. of intervals 2
Temperature .....30 °C/37 °C
Reaction slope .....Decreasing

PROCEDURE (AUTOMATED)

Refer to the appropriate instrument application guide available from us.

PROCEDURE (MANUAL)

I. SEMIMICRO METHOD

Pipette into a clean cuvette:

	TEST
Reconstituted Reagent	1.0 ml
Pre-Warm to 37°C, then add:	
Sample	0.1 ml
Mix and incubate at 37°C for 1 minute and read the absorbance at 340 nm against distilled water. Take the absorbance every minute for the next 2 min. and determine ΔA/Min.	

CALCULATIONS

ΔA/Min X 1768 = AST ACTIVITY (U/L)

Example : If the average absorbance change per minute of a sample by semimicro method is 0.028, then its AST activity would be 0.028 x 1768 = 49.5 U/L

II. MACRO METHOD

Pipette into a clean cuvette :

	TEST
ReconstitutedReagent	3.0 ml
Pre-warm to 37 °C, then add:	
Sample	0.2 ml
Mix and incubate at 37 °C for 1 minute and read the absorbance at 340 nm against distilled water (A_1). Re-incubate at 37 °C and after exactly 5 min. read the absorbance (A_2).	

NOTE : Assay can also be performed at 30 °C

CALCULATIONS

(A_1 - A_2) X 514 = AST ACTIVITY (U/L)

Example : If the A_1 of sample is 1.280 and A_2 after 5 minutes of incubation is 1.185 then its AST activity would be

(1.280 - 1.185) X 514 = 48.8 U/L

UNIT DEFINITION : One international unit (IU) is defined as the amount of enzyme that catalyzes the transformation of 1 micromole of substrate per minute under specified conditions.

SI UNITS : To convert to SI units (n Kat/L), multiply U/L by 16.67.

The following formula was used to calculate the factors for semimicro and macro methods.

AST ACTIVITY IN U/L

$$= \frac{\Delta A/min \times TV \times 1000}{\varepsilon \times SV \times d}$$

Where :

ΔA/min =Average abs. change per minute
TV = Total reaction volume (ml)
1000 = Conversion factor for U/L
ε = Molar Absorptivity of NADH at 340 nm ($6.22 \times 10^3 L \times Mole^{-1} cm^{-1}$)
SV = Sample volume
d = Light path in cm.

FOR SEMI MICRO METHOD :

$$\frac{\Delta A/min \times 1.1 \times 1000}{6.22 \times 0.1 \times 1} = \mathbf{\Delta A/min \times 1768}$$

FOR MACRO METHOD :

$$\frac{(A_1 - A_2) \times 3.2 \times 1000}{5 \times 6.22 \times 0.2 \times 1} = \mathbf{(A_1 - A_2) \times 514}$$

NOTE : If any of the test parameters have been altered, a new factor must be calculated using the above formula.

PROCEDURE NOTES

Pyridoxal phosphate can elevate AST values by activating the apoenzyme form of the transaminase. Pyridoxal phosphate may be found in diluent water contaminated with microbial growth[6].

High levels of serum pyruvate may also interfere with assay performance, Young, et al., give a list of drugs and other substances that interfere with the determination of AST activity[7]. Refer also to N.E. Saris for a list of references [8].

TEMPERATURE CONVERSION FACTOR (Tf)[3]

Assay Mixture	Tf30 °C	Tf37 °C
30 °C	1.00	1.43
37 °C	0.73	1.00

PERFORMANCE CHARACTERISTICS

COMPARISON: UDI reagent tested on MANUAL SYSTEMS (y) was compared with CAPS survey results (x). The systematic difference between the results were within CLIA specified limits.

N = 21
Correlation Coefficient 0.99
Regression Equation y = 0.93x - 2.71

PRECISION:

	Mean U/L	SD	CV%
Within run	35.6	0.52	1.45
Run to run	40.4	1.49	3.69

QUALITY CONTROL

For accuracy and precision check, we recommend the use of normal and abnormal UDI controls based on human serum.

ORDERING INFORMATION:

UDITROL 'N' (Normal Serum Control)
REF # 070N-010 2x5 ml

UDITROL'A' (Abnormal Serum Control)
REF #070A-010 2x5 ml

REFERENCES

1. Henry, J.B.: Clinical Diagnosis and Management by Laboratory Methods, W.B. Saunders and Co., Philadelphia, PA. p332-35 (1974).
2. Karmen, A. et al.: J. Clin. Invest, 34:126 (1955).
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5. Henry, R.J.: Clin, Chem. Principles and Techniques. 2nd Ed., Harper and Row, New York, p. 822 (1974).
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7. Young, D.S. et al.: Clin. Chem. 21:5 (1975).
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9. Tietz, N.W.: Fundamentals of Clinical Chemistry: W.B. Saunders Co. Philadelphia, PA p. 682 (1976).

PRODUCT AVAILABILITY

AST (SGOT) UV/KINETIC

REF # 015-120	**8 x 15 ml**
REF # 015-240	**20 x 12 ml**
REF # 015-100	**2 x 50 ml**
REF # 015-060	**5 x 12 ml**
REF # 015A-060	**20 x 3 ml**
REF # 015-050	**1 x 50 ml**

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QUALITY MANAGEMENT SYSTEM CERTIFIED

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ALKALINE PHOSPHATASE

P-NPP COLORIMETRIC/KINETIC METHOD
FOR IN VITRO DIAGNOSTIC USE

REF 004L

INTENDED USE

Quantitative determination of alkaline phosphatase (ortho Phosphoric monoester phosphohydrolase, alkaline optimum ALP; E C 3.1.3.1.) in Serum or heparinised plasma by a colorimetric kinetic method.

DIAGNOSTIC SIGNIFICANCE

Elevated levels of serum alkaline phosphatase (ALP) are observed in hepatobiliary disorders and bone disease. Highest levels of serum ALP activity are encountered in 'pagets disease' (osteitis deformans). Very high levels of ALP are present in patients with bone cancer [1]. Normal ALP levels are age dependent and are elevated during periods of active bone growth.

An increase of upto 2-3 times normal is observed in the third trimester of pregnancy. Moderate elevations of ALP (not involving the liver or bone) may be attributed to 'hodgkins' disease, congestive heart fallure, and abdominal bacterial infections [2].

Range of expected values in serum

Age	37 °C
Children 0 - 2 months	120 - 275 U/L
Children 2 - 6 months	95 - 335 U/L
Children 6 months - 3 years	120 - 275 U/L
Children 3 years - 15 years	108 - 360 U/L
Adults : Men	50 - 119 U/L
Adults : Women	43 - 110 U/L

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

METHOD PRINCIPLE

Hydrolysis of p-nitrophenyl phosphate by ALP produces inorganic phosphate and p-nitrophenol which at an alkaline pH, yields yellow p-nitrophenoxide ions. The rate of the reaction is measured by following the linear absorbance increase at 405 nm per unit time using a spectrophotometer equipped with a thermoregulated cuvette. The absorbance increase is directly proportional to the amount of ALP present in the reaction when measured within the linear reaction interval.

The UDI procedure is based upon the (optimized method of Bowers and McComb [3]).

$$O_2N\text{-}C_6H_4\text{-}O\text{-}P\text{-}O_3 + H_2O + O\bar{H} \xrightarrow{ALP} O_2N\text{-}C_6H_4\text{-}O + HPO_4 + H_2O$$

p-Nitrophenyl phosphate — Mg^{++} — p-Nitrophenoxide

REAGENTS

1. *ALKALINE PHOSPHATASE SUBSTRATE :*

5 mmol/L p-Nitrophenyl phosphate, activator and binder. Keep tightly capped and protected from light. Store at 2-8 °C

2. *ALKALINE PHOSPHATASE BUFFER:*

Buffer pH 10.2 ± 0.2, Magnesium Ions 3 mmol/L and preservative. Keep tightly capped and store at 2 - 8 °C.

PREPARATION OF WORKING REAGENT

Mix 1 volume of Alkaline phosphatase Substrate with 9 volumes of Alkaline Phosphatase Buffer. Store at 2-8 °C and stopper tightly when not in use. Stable for 21 days. For REF # 004L-060, Transfer 0.3 ml of ALP Substrate (Rgt. I) to 1 vial of ALP Buffer (2.7 ml) (Rgt. II)

STABILITY

Individual reagents are stable upto expiration date indicated on the label. Working reagent is stable for 21 days at 2 - 8 °C.

INDICATIONS OF REAGENT DETERIORATION

1. Elevated Blank Absorbance

If the absorbance of the reconstituted Alk. Phos. Rgnt. exceeds 1.4 (1-cm light path) when measured against Reagent Grade Water at 405nm, the reagent has deteriorated and should be discarded.

2. Control Assays

Fallure to obtain accurate results in the assay of freshly prepared control materials may indicate reagent deterioration.

NOTE:

UDI cannot guarantee the stability of reagents which have been:

a. transferred from their original containers.
b. improperly stored.
c. contaminated during use.

MATERIALS PROVIDED

Alkaline Phosphatase Substrate, Alkaline Phosphatase Buffer.

ADDITIONAL MATERIALS REQUIRED, BUT NOT PROVIDED

Reagent and sample pipettes, test vials, timer, spectrophotometer with thermoregulated cuvette, control sera.

SPECIMEN

SERUM OR HEPARINISED PLASMA

Hemolysis must be avoided since the concentration of ALP in red blood cells is about six times that of serum[3]. The serum should be separated from the cells within 1-2 hours after collection and stored at room temperature for a period not exceeding 2-3 hours or stored overnight at 2-8 °C. If stored for longer periods, -20 °C is recommended.

PROCEDURE PARAMETERS

Wavelength	405 nm
Reaction type	Rate
Reaction Direction	Up
Reaction Temp.	37 °C
Sample Volume	20 µl.(0.020 ml)
Reagent Volume	1.0 ml
Equilibration Time	30 Seconds
Measurement Time	60 - 180 seconds (not to exceed 360s)
Factor	2720

PROCEDURE (AUTOMATED)

Refer the appropriate instrument application manual available from us.

PROCEDURE (MANUAL)

pipette into a clean cuvette:

	TEST
Working Reagent	1.0 ml
Pre-warm at 37 °C and add :	
Sample	0.02 ml
Mix and aspirate into the thermo-regulated flow cell set at 37 °C. Allow 30-60 seconds for temperature equilibration and read absorbance (at 405 nm against distilled water) every minute for 3 minutes to determine ΔA/Min.	

CALCULATION OF RESULTS:

Multiply ΔA/Min with a factor of 2720 to get results in U/L .
EXAMPLE: Assume that ΔA/Min of a sample was 0.035, 0.036 over a period of 3 minutes. The average ΔA/Min is 0.0355, So that ALP Activity
(U/L) = ΔA/Min x 2720 = 0.0355 x 2720 = 97 U/L.

ALTERNATE PROCEDURE

(Applicable to spectrophotometers requiring final volume greater than 1.0 ml)

Pipette into a clean cuvette:

	TEST
Working Reagent	2.5 ml
Pre-warm at 37 °C and add :	
Sample	0.05 ml
Mix and incubate at 37 °C for exactly 1 min. and read absorbance A_1 at 405 nm against distilled water and return to incubator maintained at 37 °C for exactly 2 min. and read absorbance A_2 at 405 nm against distilled water.	

CALCULATION OF RESULTS :

$$\Delta A/Min. = \frac{A_2 - A_1}{2}$$

Multiply ΔA/Min. with a factor of 2720 to get results in U/L.

EXAMPLE : Assume that A1 of sample is 0.143 and A2 is 0.214. So that :

$$\Delta A/Min = \frac{0.214 - 0.143}{2} = 0.0355$$

ALP activity (U/L) = ΔA/Min x 2720 = 0.0355 x 2720 = 97 U/L.

UNIT DEFINITION: One unit (U) is the amount of enzyme that will transform one micromole of substrate under the specified conditions of the reaction. The following formula was used to derive the factor for calculating ALP activity in U/L:

$$U/L = \frac{\Delta A/Min \times TV \times 10^6}{\varepsilon \times SV \times L}$$

Where ΔA/Min = Measured rate of change in absorbance per minute

TV = Total volume of reaction mixture (ml)

10^6 = Factor to convert moles to micromoles

ε = Molar absorptivity of p-nitrophenol at 405 nm (18.75×10^3 L x $mole^{-1}$ cm^{-1})

SV = Sample Volume (ml)

L = Light path (cm)

For the above procedure :

$$U/L = \frac{\Delta A/Min \times 1.020 \times 10^6}{18.75 \times 10^3 \times 0.020 \times 1} = \Delta A \text{ min} \times 2720$$

FOR ALTERNATE PROCEDURE:

$$U/L = \frac{\Delta A/Min \times 2.250 \times 10^6}{18.75 \times 10^3 \times 0.050 \times 1} = \Delta A \text{ min} \times 2720$$

PERFORMANCE CHARACTERISTICS

1. LINEARITY AND SENSITIVITY: ALP values upto 700 U/L can be measured with this method. Typically, 0.001 A represents about 2.7 U/L when measured in a 1 - cm light path at 405 nm.

2. COMPARISON : UDI reagent tested on MANUAL SYSTEMS (y) was compared with CAPS survey results (x). The systematic difference between the results were within CLIA specified limits, N = 24

Correlation Coefficient 0.981

Regression Equation : $y = 1.1x + 11$

3. **PRECISION:**

	Mean U/L	SD	CV%
Within run	287.4	7.81	2.72
Run to run	101.2	3.10	3.06

PROCEDURE NOTES

1. If the ΔA/min is greater than 0.257 repeat the test using a 1:10 dilution of the serum with 0.85% sodium chloride (physiological saline); multiply result by 10.

2. For a comprehensive review of possible ":in vitro" and "in vivo" drug, metabolite, and disease effects on ALP, refer to (4 and 5)

3 If "U/L" is to be converted to SI units (nKat/L), use the conversion factor: U/L = 16.67 nKat/L.

QUALITY CONTROL

For accuracy and precision check, we recommend the use normal and abnormal UDI controls based on human serum.

ORDERING INFORMATION:

UDITROL 'N' (Normal Serum Control)
REF # 070N-010 2x5 ml

UDITROL 'A' (Abnormal Serum Control)
REF # 070A-010 2x5 ml

REFERENCES

1. Kochmar, J.F. & Moss, D.W; Fundamentals of Clinical chemistry, N.W. Tietz (ed), P. 604-609, W.B Saunders and Company, Philadelphia, PA (966).
2. Kaplan, M.M.: New England J. Med. 286:200 (1972).
3. Bowers, GN Jr., and McComb, RB; A continuuous spectrophotometric method for measuring the activity of serum alkaline phosphatase. Clin. Chem. 12:70 (1966).
4. Young, DS et al, Clin Chem, 21:1D (1975).
5. Friedman, RB et al, Clin Chem. 26:1D (1980).

PRODUCT AVAILABILITY

ALKALINE PHOSPHATASE (Color/Kinetic)

REF # 004L-250	**5 X 50 ml**
REF # 004L-060	**20 X 3 ml**
REF # 004L-050	**1 X 50 ml**

EC REP
(Authorized representative in the European Community)

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ACID PHOSPHATASE REAGENT SET

(TOTAL, PROSTATIC AND NON-PROSTATIC)
(COLOR/KINETIC METHOD)

REF 002

FOR IN VITRO DIAGNOSTIC USE

INTENDED USE

For the quantitaive determination of acid phosphatase (Total, Prostatic and Non-prostatic) in serum by α-naphthylphosphate colorimetric kinetic method.

DIAGNOSTIC SIGNIFICANCE:

Non-specific acid phosphatase activity is widely distributed throughout the living world. This enzyme secreted by the human prostate gland has attracted most attention, because of its clinical importance, and extensive characterization and structural studies have now been carried out on it. Large elevations of prostatic acid phosphatase are found in cases of metastasized prostatic cancer. Since acid phosphatase is also produced in other tissues, the prostatic isoenzyme must be distinguished from the non-prostatic for accurate diagnosis. Elevated levels of non-prostatic acid phosphatase have been observed in patients with Paget's disease, hyperparathyroidism with skeletal involvement, and in cancers which have invaded the bones[1, 2].

RANGE OF EXPECTED VALUES[3]

Total Acid Phosphatase (37 °C) 0:0 - 12 U/L
Prostatic Acid Phosphatase (37 °C) 0.0 - 4.0 U/L

Values were taken from literature[3]. It is strongly recommended that each laboratory should establish its own normal range.

PRINCIPLE

α- Naphthylphosphate + H_2O $\xrightarrow{ACP}$ α-Naphthol + Inorg. Phos.
α-Naphthol + Fast Red TR $\longrightarrow$ Diazo Dye (Chromophore)

The α-naphthol released from the substrate α-naphthylphosphate by acid phosphatase is coupled with Fast Red TR to produce a colored complex which absorbs light at 405 nm. The reaction can be quantitated photometrically because the coupling reaction is instantaneous.

L-Tartrate inhibits prostatic acid phosphatase but does not interfere with the reaction mechanism. Therefore, if testing is performed in the presence and in the absence of L-Tartrate, the difference between the results of the two assays is the level of prostatic acid phosphatase in the serum.

REAGENTS

1. Acid phosphatase Buffer : *Citrate Buffer 60 mol pH 5.3 ± 0.1
2. Acid phosphatase Reagent: (Concentrations refer to reconstituted reagent) α-Naphthylphosphate 3 mmol, Fast Red TR salt 1.5 mmol/L.
3. L-Tartrate Reagent: Sodium L-Tartrate 2 mol, Citric Acid 70 mmol, Sodium Citrate 10 mmol, pH 5.3 ± 0.1.
4. Acetate Buffer: 5 mol, pH 5.0.

PRECAUTIONS

Reagents are for In-Vitro Diagnostic use only.

REAGENT PREPARATION

1. Reconstitute acid phosphatase reagent with volume of buffer Stated on the label. Swirl to dissolve.
2. L-Tartrate Reagent is ready to use. Warm reagent to aid in dissolution, if necessary.
3. Acetate buffer is ready to use.

REAGENT STORAGE AND STABILITY

1. Unopened vials are stable until stated expiration date on vial label when stored refrigerated 2 °C to 8 °C.
2. The reconstituted acid phosphatase reagent is stable for one day at room temperature (15 °C to 25 °C) and for seven days when stored refrigerated at 2 °C to 8 °C.
3. The L-Tartrate Reagent is stable refrigerated 2 °C to 8 °C until expiration date on vial label. If crystallization occurs, warm at moderate temperature (40 °C to 50 °C) until dissolved.
4. Acetate Buffer solution is stable refrigerated 2 °C to 8 °C until the expiration date listed on the vial label.

REAGENT DETERIORATION

The reagent should not be used if:

1. The reconstituted acid phosphatase reagent, without serum added, has an absorbance greater than 0.500 when measured at 405 nm against water.
2. If precipitated L-Tartrate Reagent does not dissolve even after warming at 40 °C to 50 °C.

SPECIMEN COLLECTION AND STORAGE

1. Use only clear, unhemolyzed serum.
2. Serum must be separated from clot within two hours after collection.
3. Acid Phosphatase activity is extremely labile at room temperature. Stabilization of the enzyme can only be achieved by acidifying with the Acetate Buffer provided. Add 20 µl (0.02 ml)of buffer per 1.0 ml of serum. Mix. Treated serum samples will remain stable for seven days when kept refrigerated at 2 °C to 8 °C[4].
4. **Do not use plasma**. Some anticoagulants inhibit acid phosphatase activity and/or cause turbidity[5].

MATERIALS REQUIRED BUT NOT PROVIDED

1. Test tubes/rack.
2. Accurate pipetting devices.
3. Distilled/Deionized water.
4. Timer.
5. Spectrophotometer capable of reading at 405 nm.
6. Temperature must be closely controlled during assay. A temperature controlled (37 °C) spectrophotometer cuvette should be used.

PROCEDURE (AUTOMATED)

Refer to appropriate instrument application instructions.

PROCEDURE (MANUAL)

Note: Stabilize acid phosphatase immediately after separation of the serum from the clot by adding 20 µl (0.02ml) of Acetate Buffer per 1.0 ml of serum. Mix and store in refrigerator until assay is ready to be performed.

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A. TOTAL ACID PHOSPHATASE

Pipette into a clean cuvette:

	TEST
Reconstituted Acid Phos. Rgt.	1.0 ml
Set the cuvette temperature at 37 °C and then add:	
Sample	0.1 ml
Mix, incubate at 37 °C for 5 min. and then read the absorbance at 405 nm against dist. water every minute for 5 min. to determine ΔA/Min.	

CALCULATIONS:

Total Acid Phosphatase activity **(U/L) = ΔA/min. x 853**

B. NON-PROSTATIC ACID PHOSPHATASE

Pipette into a clean cuvette:

	TEST
Reconstituted Acid Phos. Reagent L. Tartrate reagent	1.0 ml 0.01 ml
Mix, set the cuvette temperature at 37 °C and then add:	
Sample	0.1 ml
Mix, incubate at 37 °C for 5 min. and then read the absorbance at 405 nm against dist.water every minute for 5 min. to determine ΔA/Min.	

CALCULATIONS:

Non-Prostatic Acid Phosphatase activity **(U/L) = ΔA/min. x 860**

C. PROSTATIC ACID PHOSPHATASE

The value is obtained by subtracting the result of non-prostatic acid phosphatase assay (B) from the total acid phosphatase assay (A).

UNIT DEFINITION

One Unit is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under defined conditions. The following formula has been used to derive the factors for caculations

A. Total Acid Phosphatase:

$$\frac{\Delta A/MIN. \times 10^6 \times 1.1}{12.9 \times 10^3 \times 1.0 \times 0.1} = U/L = \mathbf{\Delta A/min \times 853}$$

B. Non-prostatic Acid Phosphatase:

$$\frac{\Delta A/MIN. \times 10^6 \times 1.11}{12.9 \times 10^3 \times 1.0 \times 0.1} = U/L = \mathbf{\Delta A/min \times 860}$$

Where:

10^6	=	Conversion of moles to micromoles.
1.1	=	Total reaction volume (Total AcP)
1.11	=	Total reaction volume (Non-Prost. AcP)
12.9×10^3	=	Molar absorptivity of α-naphthol-Fast Red TR Complex at 405 nm.
1.0	=	Light path in cm.
0.1	=	Sample volume (ml).

SAMPLE CALCULATIONS

ΔA/MIN. Total acid phosphatase = 0.01
ΔA/MIN. Non-Prostatic acid phosphatase = 0.009
Total acid phosphatase: 0.01 x 853 = 8.5 U/L
Non-Prostatic acid phosphatase: 0.009 x 860 = 7.7 U/L
Prostatic Acid phosphatase: 8.5 - 7.7 = 0.8 U/L

LIMITATIONS

Samples with values above 75 U/L at 37 °C should be diluted 1:9 with normal saline, re-run, and the final results to be multiplied by 10.

INTERFERENCES

1. High levels of bilirubin (Icteric Samples) reportedly inhibit acid phosphatase activity determined by this procedure[6].
2. A number of drugs and substances affect Acid Phosphatase activity. Young et al[7] has published a comprehensive list.

PERFORMANCE

1. Linearity : 75 U/L at 37 °C.
2. Comparison : A study performed using this method with a commercial reagent with a similar formulation yielded the following : N = 22.

	Total	Prostatic
Correlation Coefficient	0.97	0.98
Regression Equation	Y = 0.96X + 0.38	Y = 0.97X - 0.23

3. Precision : With in Run (N = 20)

	Total Acid Phosphatase	
Mean (U/L)	8.9	19.0
S.D.	0.7	1.4
C.V. %	7.8	7.4

Run to Run (N = 15)

	Total Acid Phosphatase	
Mean (U/L)	10.2	20.2
S.D.	1.2	1.5
C.V. %	11.7	7.4

QUALITY CONTROL

For accuracy and precision check, we recommend the use of normal and abnormal UDI controls based on human serum.

ORDERING INFORMATION:

UDITROL 'N' (Normal Serum Control) REF # 070N-010 2x5 ml
UDITROL 'A' (Abnormal Serum Control) REF # 070A-010 2x5 ml

REFERENCES:

1. Bergmeyer, H.V., Methods of Enzymatic analysis. Weinheim, Verlag chemie, 3rd p.92 (1984).
2. Tietz, N.W., Fundamentals of Clinical Chemistry. Philadelphia, W.B. Saunders, p.614 (1976).
3. Tietz, N.W., Fundamentals of Chlinical Chemistry., Philadelphia, W.B. Saunders, p. 618 (1976).
4. Ellis, G. et al, J. Clin. Path. 24:493 (1971).
5. Henry, R.J., Clin. Chem. Prin. and Tech., Hoeber, New York (1964).
6. Shaw, L.M. et al, Am. J. Clin Path. 68:57 (1977).
7. Young, D.S. et al, Clin. Chem. 21:No. 5 (1975).

PRODUCT AVAILABILITY

ACID PHOSPHATASE REAGENT SET (COLOR/KINETIC)

REF # 002-060	**10 x 6 ml**
REF # 002-030	**5 x 6 ml**
REF # 002-006	**1 x 6 ml**

EC REP
(Authorized representative in the European Community)

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GAMMA - GT (KINETIC)

REF 035

FOR IN VITRO DIAGNOSTIC USE

INTENDED USE:

Quantitative determination of Gamma Glutamyl Transpeptidase (4-Glutamyl Transferase,GGT: EC2.3.2.2) in serum or plasma by the colorimetric kinetic method.

DIAGNOSTIC SIGNIFICANCE:

Elevated GGT levels are associated with: Viral Hepatitis, chronic, active and toxic hepatitis, cirrhosis of liver, biliary cirrhosis, acute pancreatitis, chronic pancreatitis with biliary tract involvement. malignant neoplasm of liver and pancreas, alcoholism, nephrotic syndrome, acute myocardial infarction, acute cholecystitis, cholangitis, infectious mononucleosis and diabetes mellitus.

Till now no diagnostic significance is known for decreased GGT levels.

For a more comprehensive review of disease effects on clinical laboratory tests, see reference [1].

Range of expected values in serum

Temperature	25 °C	30 °C	37 °C
Men	6 - 28 U/L	8 - 37 U/L	10 - 47 U/L
Women	4 - 18 U/L	5 - 24 U/L	7 - 30 U/L

It is strongly suggested that each laboratory should establish its own normal range

METHOD PRINCIPLE:

GGT catalyses the transpeptidation of the gamma glutamyl group of gamma glutamyl-p-nitroanilide (L-GGPNA) to glycylglycine, yielding free p-nitroaniline which absorbs at 405 nm. Enzyme activity is proprtional to the increase in absorbance at 405 nm. The UDI procedure has been optimised according to Szasz and Rosalki[2, 3], and is performed as kinetic method.

GGPNA + Glycylglycine $\xrightarrow{\gamma\text{-GT}}$ P-nitroaniline + γ-glutamyl glycylglycine.

REAGENTS

1. ***GGT REAGENT (Soluble)*** : (Concentrations refer to reconstituted reagent) Gamma glutamyl p-nitroanilide 4.79 mmol, Glycyl Glycine 100 mmol, Tris 180 mmol, pH 8.2 ± 0.1

   Store at 2 °C to 8 °C. Must be kept tightly capped and protected from excessive light and moisture. Stable until expiration date indicated on the vial label.

   RECONSTITUTION

   Reconstitute 1 vial of γ-GT reagent with the volume of distilled water specified on the label. Reconstituted reagent is stable for 5 days at 2 °C to 8 °C and 8 hours at room temp (15 °C to 25 °C)

NOTE : The reagent may have to be re-heated after refrigeration to re-dissolve the substrate.

CHEMICAL PRECAUTIONS

p-Nitroaniline is highly toxic and may be absorbed through the skin. For other reagents exercise the normal precautions required for the handling of all laboratory reagents. Pipetting by mouth is not recommended for any laboratory reagent.

INDICATIONS OF REAGENT DETERIORATION

1. Physical appearance
   Darkening of the reagent, appearance of turbidity, or crystal formation that will not readily dissolve are signs of possible reagent deterioration.

2. Control assays
   Failure to obtain accurate results in the assay of control material may indicate reagent deterioration.

3 If the reconsitituted reagent's absorbance against distilled water is greater than 0.550 which may indicate reagent deterioration.

NOTE:

UDI cannot guarantee the stability of reagents which have been
   a) Transferred from their original containers.
   b) Improperly stored
   c) Contaminated during use.

SPECIMEN

SERUM OR EDTA PLASMA

Serum GGT is reportedly [2,4] stable for at least 8 hours at room temp. (15 °C to 25 °C) , 3 days at 2 °C to 8 °C, or one week at -20 °C.

Avoid the use of hemolysed specimen.

MATERIALS REQUIRED , BUT NOT PROVIDED

Reagent and sample pipettes, test vials or cuvettes, timer, test tube rack, 37 °C heating bath, spectrophotometer, control serum.

PROCEDURE PARAMETERS

Wavelength	405 nm (400 - 420 nm).
Reaction Type	Delta Kinetic with factor.
Units	U/L.
Factor	2121.
Incubation Time	30 Seconds.
Interval Time	60 Seconds.
Number of Intervals	2.
Temperature	25 °C / 30 °C / 37 °C.
Reaction Slope	Increasing.

PROCEDURE (AUTOMATED)

Refer to appropriate instrument application manual available from us.

PROCEDURE (MANUAL)

Pipette into clean cuvette:

	Test
Reconstituted GGT Reagent	1.0 ml
Pre-warm at 25 °C / 30 °C / 37 °C, for 3-5 minutes and add:	
Sample	0.05 ml
Mix and incubate for 30 seconds at 25 °C / 30 °C / 37 °C and read the absorbance at 405 nm against distilled water. Continue at exactly 1 minute interval for the next two minutes and calculate ΔA/min.	

CALCULATIONS

A/min x 21Δ21 = GGT Activity in TEST (U/L)

The following formula has been used to derive the calculation of factor

$$\frac{\Delta A/min. \times 10^3 \times T.V. \times 1000}{\Sigma \times S.V \times L} = U/L$$

ΔA/min = mean absorbance change over 1- min. interval
T.V. = total volume (ml)
Σ = molar absorpitivity of p-nitroaniline at 405 nm = 9.9×10^3 liters/mole x cm
SV = sample volume (ml)
L = light path (1 cm)

$$\frac{\Delta A/min. \times 10^3 \times 1.05 \times 1000}{9.9 \times 10^3 \times 0.05 \times 1} = \Delta A/min \times 2121$$

EXAMPLE : A sample demonstrated an absorbance change of 0.025 over a 1 min. interval. Then 0.025 x 2121 = 53 U/L

DEFINITION OF ENZYME UNIT

One international unit (IU) of GGT activity is the amount of enzyme which transfers 1 µmol of glutamate per minute per liter of sample with the concurrent release of 1µmol of p-nitroaniline under the specified conditions of the procedure.

PERFORMANCE CHARACTERISTICS

1. COMPARISON : UDI reagents tested on MANUAL SYSTEMS(y) was compared with similar UDI reagent for other systems (x) which in turn is matching with CAPS survey results. The reagent was also compared with another commercial reagent. The systematic difference between the results were within CLIA specified limits, N = 30

Correlation Coefficient 1.0
Regression Equation y = 0.98x - 1.05

2. PRECISION:

	Mean U/L	SD	CV%
Within run	56.4	1.43	2.54
Run to run	152.5	7.27	4.76

LINEARITY AND SENSITIVITY

The method is linear up to 250 U/L. Typically, 0.001A corresponds to about 2.1 U/L concentration of GGT under the specified conditions of the test.

PROCEDURE NOTES

1. This procedure measures GGT activity irrespective of its tissue organ of origin.
2. Interfering substances: BSP may inhibit GGT activity[2]. Alcohol[5] Barbiturates and antiepileptic drugs, i.e. phenytoin[6] reportedly increase serum GGT levels. For a more comprehensive review of drug effects on clinical laboratory test, see reference[7].
3. If the linearitry of the method is exceeded (due to reagent exhaustion or photometric capability), re-run the test using 25 µl of sample and multiply the result by 2, or use 10µl of sample and multiply result by 5.

QUALITY CONTROL

For accuracy and precision check, we recommend the use of normal and abnormal UDI controls based on human serum.

ORDERING INFORMATION:
UDITROL 'N' (Normal Serum Control) REF # 070N-010 2x5 ml
UDITROL 'A' (Abnormal Serum Control) REF # 070A-010 2x5 ml

REFERENCES

1. Friedman, RB et al, Clin. Chem, 26: 106D-107D(1980).
2. Szasz, G. Clin. Chem. 15:24 (1969).
3. Rosalki ,S et al, Ann Clin Biochem, 7:143(1970).
4. Zein, M and Discombe, G, Lancet, 2:748(1971).
5. Rosalki, SB and Rau, D, Clin. Chim. Acta, 39:41(1972).
6. Whitfield, JB et al, Gut, 13: 702(1972).
7. Young, DS et al, Clin Chem, 21:1 D (1975).

PRODUCT AVAILABILITY

GAMMA-GT (KINETIC)

REF # 035-150	**15 x 10 ml**
REF # 035-060	**20 x 3 ml**

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AMYLASE (COLOR/KINETIC)

REF 010

FOR IN VITRO DIAGNOSTIC USE

INTENDED USE

For the quantitative determination of amylase in serum, heparinized plasma and urine.

DIAGNOSTIC SIGNIFICANCE

The determination of amylase activity in serum and urine is most commonly performed for the diagnosis of acute pancreatitis. In acute pancreatitis, amylase levels are elevated for longer periods of time in urine than in serum. Therefore, determining the ratio of the amylase and creatinine clearances is important in following the course of the pancreatitis[1].

RANGE OF EXPECTED VALUES[2]

Serum : 16-108 U/L Urine: 0 - 14 U/Hour
Since the expected values are affected by age, sex, diet and geographical location, each laboratory is strongly urged to establish its own normal range.

METHOD PRINCIPLE

Wallenfels et al[3] introduced p-Nitrophenylglycosides as defined substrates for amylase determination in a procedure that eliminated interference from endogenous glucose and pyruvate. The present procedure is based on modification of Wallenfels, using as substrate p-Nitrophenyl-D-maltoheptoside (PNPG7) with the terminal glucose blocked to reduce spontaneous degradation of the substrate by glucosidase and glucoamylase[4]. The test is performed in a kinetic mode with a very short lag time and offers much greater stability than previous amylase methodologies.

Amylase hydrolyzes p-nitrophenyl D-maltoheptoside (PNPG7) to p-nitrophenylmaltotriose (PNPG3) and maltotetrose.

Glucoamylase hydrolyzes PNPG3 to p-Nitrophenylglycoside (PNPG1) and glucose. Then PNPG1 is hydrolysed by glucosidase to glucose and p-nitrophenol which produces a yellow color. The rate of increase in absorbance is measured at 405 nm and is proportional to the amylase activity in the sample.

PNPG7 $\xrightarrow{\text{AMYLASE}}$ PNPG3 + Maltotetrose
PNPG3 $\xrightarrow{\text{GLUCOAMYLASE}}$ PNPG1 + Glucose
PNPG1 $\xrightarrow{\text{GLUCOSIDASE}}$ p-Nitrophenol + Glucose

REAGENTS

1. AMYLASE SUBSTRATE (PNPG7): (Concentrations refer to reconstituted reagent) p-Nitrophenyl D-Maltoheptoside 0.9 mM, Glucosidase 25,000 U/L, Glucoamylase 10,000 U/L, Sodium Chloride 50 mM, Calcium Chloride 5 mM and Buffer 50 mM, pH 6.9 ± 0.01.

RECONSTITUTION

Reconstitute reagent with the volume of distilled water stated on the vial label.

PRECAUTION

DO NOT PIPETTE WATER BY MOUTH to avoid contamination with salivary amylase.

REAGENT STORAGE & STABILITY

1. Store dry reagent at 2-8 °C. Stable up to expiration date indicated on vial label.
2. Reconstituted reagent is stable for at least one day at room temperature (18-25 °C) and at least 14 days when refrigerated (2-8 °C).

REAGENT DETERIORATION

Do not use reagent if:
1. The absorbance of the reagent is greater than 0.70 when measured at 405 nm against water in a cuvette with a 1 cm path length.
2. The reagent fails to meet the stated parameters of performance.

SPECIMEN

SERUM / HEPARINIZED PLASMA / URINE
Anticoagulants, such as Citrate and EDTA, bind calcium, anion, needed for amylase activity. Therefore, plasma with any anticoagulant other than heparin should not be used.

Urine specimen should be adjusted to a pH of 7 and kept refrigerated until assayed.

Amylase in serum and urine is reported as stable for one week at room temperature (18-25 °C) and protected against evaporation and bacterial contamination[5].

MATERIALS PROVIDED

AMYLASE SUBSTRATE (PNPG7)

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

1. Accurate pipetting devices.
2. Test tubes / rack
3. Timing device.
4. Heating block /bath (37 °C).
5. Spectrophotometer capable of reading at 405 nm (400-420 nm). The cuvette compartment should be temperature controlled to maintain temperature (37 °C) during the assay.

PROCEDURE (AUTOMATED)

Refer the appropriate instrument application manuals available from us.

	TEST
Reconstituted amylase Reagent	1.0 ml
Pre-warm at 37oC for 5 minutes and add:	
Sample	0.025 ml
Mix and incubate at 37oC for 90 seconds and read the absorbance at 405 nm against distilled water. Continue readings every 30 seconds for 2 minutes and determine ΔA/Min.	

PROCEDURE (MANUAL)

Pipette into clean dry test tubes:

CALCULATIONS

ΔA/Min x 4824 = Amylase Activity in TEST (U/L)

EXAMPLE: If ΔA/min = 0.03 then 0.03 x 4824 = 145 U/L.

SI Units

To convert into SI Units (nKat/L) multiply the U/L value by 16.67

INTERFERENCES

A number of drugs and substances affect the determination of amylase[2, 6]. Young et al have published a comprehensive list of such substances[7].

LIMITATIONS

Samples that exceeded the linearity limit (2,000 U/L) should be diluted with an equal volume of saline and re-run. Multiply the results by two.

PERFORMANCE

1) LINEARITY : 2,000 U/L.

2. COMPARISON : UDI reagent tested on Manual Systems(y) was compared with CAPS survey results(x). The systematic difference between the results were within CLIA specified limits. N = 25

Correlation Coefficient	0.991
Regression Equation	y = 1.1x + 6.7

3) PRECISION:

	Mean (U/L)	S.D.	C.V.%
Within run	116.6	9.52	8.17
Run to run	80.4	4.55	5.66

QUALITY CONTROL

For accuracy and precision check, we recommend the use of normal and abnormal UDI controls based on human serum.

ORDERING INFORMATION:

UDITROL 'N'(Normal Serum Control)
REF#070N-010 2x5 ml

UDITROL 'A' (Abnormal Serum Control)
REF # 070A-010 2x5 ml

BIBLIOGRAPHY

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PRODUCT AVAILABILITY

AMYLASE (Color/Kinetic)

REF # 010-060 12 x 5 ml

EC REP
(Authorized representative in the European Community)

mdi Europa GmbH
Wittekamp 30
D-30163 Hannover
Germany

CE

QC 011/010 Issue 07, Issued on April 2008. EDMA CODE 11.01.01.07.00

BM TEST G6PD Deficiency

Screening Test

mannheim boehringer

Procedure according to Beutler[1]

Cat. No. 166 537 for 3 x ca. 240 tests

The enzyme determined is glucose-6-phosphate dehydrogenase (EC 1.1.1.49), which is abbreviated either as G6P-D or G6P-DH.

Test principle

$$\text{glucose-6-P} + \text{NADP}^+ \xrightleftharpoons{\text{G6P-DH}} \text{gluconate-6-P} + \text{NADPH} + \text{H}^+$$

The NADPH produced in the reaction fluoresces under long-wave UV-light. If there is a marked deficiency of G6P-DH, or if this enzyme is lacking entirely, no fluorescence will be observed.

Sample material

(Cf. notes 1 and 2.)

– Whole blood dried on filter paper[2].

Apply a drop of blood to absorbent paper ("Guthrie Test Paper", Schleicher & Schuell No. 2992) and let dry completely (stable for one week at +20 to 25° C).

– Whole blood.

Whole blood may be used instead of dried blood. Heparin, citrate, oxalate, and EDTA are suitable anticoagulants. The blood specimens are stable for seven days at most. Use 0.005 ml for the assay.

Reagents

Contents of solution	Concentrations in the test
glucose-6-P	1 mmol/l
NADP	0.75 mmol/l
GSSG (oxidized glutathione)	0.8 mmol/l
saponin	0.2 %
tris(hydroxymethyl)-aminomethane	225 mmol/l, pH 7.8

Preparation and stability of reagent solution

Dissolve substrate in bottle 1 by adding the contents of bottle 2 up to the mark.

Stable for four weeks at + 4° C
three months at –20° C.

Sample preparation

Punch out a disk of blood-stained paper of 5 mm diameter (3 mm can also be used).

Procedure

Introduce into a vial (volume 1–3 ml):	
1 blood disk	5 mm ∅
reagent solution	0.1 ml
Mix well, incubate for 10 min at 25° C, and then apply ca. 0.01 ml of test solution to the enclosed filter paper.	

Evaluation

When the filter paper is dry (ca. 1 hour), view under a long-wave UV-lamp in a darkened room (cf. note 3).

Specimens obtained from patients with normal or just slightly depressed G6P-DH activity will show strong fluorescence. Failure to fluoresce after 10 minutes' incubation suggests a total lack or marked deficiency of G6P-DH.

Please note

1. In some forms of G6P-DH deficiency, young erythrocytes manifest normal enzyme activity. Blood from patients who have just experienced a hemolytic crisis must first be treated by the procedure of Herz *et al.*[3] to separate the older erythrocytes from the prevailing population of young ones. Use 0.005 ml of the suspension so obtained for the assay.
2. If the patient has received a blood transfusion, this test is clinically significant only after 30 days have elapsed, as the donor's erythrocytes generally manifest a normal G6P-DH activity and can thus bias the result before the expiration of this time.
3. Commercially available UV-lamps emitting long-wave UV-light are adequate for the evaluation.

Reagent 2 contains sodium azide as stabilizer. Do not swallow. Avoid contact with the skin and mucous membranes.

References

1 Beutler, E., and M. Mitchell (1968). *Blood* **32**: 816.

2 Dow, P. A., et al. (1974). *Amer. J. Clin. Path.* **61**: 333.

3 Herz, F., et al. (1970). *Blood* **35**: 90.

UDI

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LIPASE REAGENT SET

(TURBIDIMETRIC)

REF 049

FOR IN VITRO DIAGNOSTIC USE

INTENDED USE

Quantitative turbidimetric determination of pancreatic lipase (Triacylglycerol acyl-hydrolase, EC 3.1.1.3) in serum

DIAGNOSTIC SIGNIFICANCE

The measurement of lipase activity in serum and other body fluids to evaluate conditions associated with pancreas[1].

RANGE OF EXPECTED VALUES[4]

Serum

Adults	10-150 U/L
Old individuals (more than 60 years)	18-180 U/L

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

METHOD PRINCIPLE

Serum is incubated at 37 °C with an olive oil substrate buffered at pH 9.0. Hydrolysis of triglycerides present in the olive oil by pancreatic lipase causes a decrease in the turbidity of the reaction mixture. The decrease in absorbed light at 400 nm is measured over a period of 5 minutes and reflects the activity of lipase in the sample.

The UDI Lipase procedure is a modified Vogel-Zieve turbidimetric method.

Triglycerides $+H_2O \xrightarrow{\text{Lipase}}$ > mono + di-Glycerides +Fatty acids

REAGENTS

1. ***LIPA-ZYME SUBSTRATE:*** 0.8% w/v Olive Oil in Ethanol. Must be kept tightly capped and protected from evaporation.
2. ***LIPA-ZYME BUFFER*** :(Concentration based upon reconstitution) 70 mmol/L Tris (hydroxymethyl) aminomethane, 8.7 mmol/L Sodium deoxycholate and Preservative; pH 9.3 ± 0.05 at 25 °C. Protect from contamination.

RECONSTITUTION

Reconstitute each vial with 30 ml of Reagent Grade water and swirl to mix. Date bottle and store at 2-8 °C. Use within 7 days.

3. LIPA-ZYME PEG REAGENT: 20% w/v Polyethylene glycol 6000; Buffered and Stabilized. Keep tightly capped and protected from contamination.

STORAGE AND STABILITY

Store all reagents included in this reagent set at 2 - 8 °C. All reagents are stable up to expiration date indicated on the label.

CHEMICAL PRECAUTIONS

Exercise the normal precautions required for the handling of laboratory reagents. Pipetting by mouth is not recommended for any laboratory reagent.

INDICATIONS OF REAGENT DETERIORATION

1. Physical appearance

LIPA-ZYME SUBSTRATE should remain clear. If turbidity is observed prior to use in preparation of the working reagent, the reagent may be contaminated and should be discarded.

2. Control assay

Failure to obtain accurate results in the assay of control materials may indicate reagent deterioration.

NOTE: UDI cannot guarantee the stability of reagents which have been:

a. transferred from their original containers
b. improperly stored
c. contaminated during use.

SPECIMEN

SERUM

Serum should be removed from the cells soon after drawing (no longer than 2 hours).

Lipase activity in serum is reportedly[4] stable at room temperature for a week, at 2-8 °C for 3 weeks and for several months at -20 °C. Bacterial contamination may result in an increase in lipase activity. Serum should be free from hemolysis as hemoglobin reportedly [5] inhibits "pancreatitis lipase".

MATERIALS PROVIDED

Lipa-zyme Substrate, Lipa-zyme Buffer and Lipa-zyme PEG Reagent.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

Sample and reagent pipettes, test vials or cuvettes, timer 37 °C heating bath, control serum, spectrophotometer, 50 ml Erlenmeyer flask or reagent bottle.

PREPARATION OF WORKING REAGENT

Using a volumetric pipette, add 1.0 ml of lipa-zyme substrate to 30 ml of reconstituted lipa-zyme buffer. The tip of the pipette must be kept below the surface of the buffer until completely empty. Swirl the vessel during the addition and after the addition. This working reagent is stable for 7 days at 2-8 °C, when it is tightly capped and protected from contamination.

PROCEDURE (MANUAL)

Pipette into clean dry test tubes:

	TEST	BLANK
Working Reagent	3.0 ml	3.0 ml
Pre-incubate for 5 minutes at 37 °C. Add (using timed intervals)		
Sample	0.1 ml	--
Read the absorbance (Ao) immediately at 400 nm against distilled water. Then transfer to water bath at 37 °C. Read absorbance (A1) after exactly 5 minutes at 400 nm against distilled water.		

CALCULATION OF RESULTS

$$\frac{\text{TEST(Ao - A1)- BLANK (Ao - A1)}}{\text{BLANK (Ao)}} \times 3000 = \text{LIPASE ACTIVITY IN U/L}$$

EXAMPLE

$$\frac{(0.784 - 0.770) - (0.706 - 0.705)}{0.706} \times 3000 = 55 \text{ U/L}$$

PERFORMANCE CHARACTERISTICS

Within run Precision was based upon assaying normal and abnormal sera for 5 times. The results are as follows:

MEAN VALUE (U/L)	STD. DEV.	CV%
34	2.5	7.4
129	3.1	2.4

Between run precision was based upon assaying normal and abnormal sera for 5 times. The results are as follows:

MEAN VALUE (U/L)	STD. DEV.	CV%
37	6.2	16.7
125	7.7	6.2

SENSITIVITY

Typical sensitivity for the method measured at 400 nm in a 1-cm light path is 0.001A = 3 U/L.

LINEARITY

The UDI lipase procedure is linear up to 280 U/L when performed according to the above directions. Values above this concentration should be re-run on dilution with distilled water and the results to be multiplied by an appropriate dilution factor.

PROCEDURAL LIMITATIONS

1. Quality Control Materials
   Some commercial quality control sera may contain lipase of an animal source that may show difference in assay values with this method. A check with the manufacturer of the control sera will confirm the lipase source.
2. Moderate to extreme lipemia may require the dilution of the sample with distilled water. A 1:5 dilution is usual. Re-run the assay and multiply the results by 5.
3. For a comprehensive review of drug and disease effects on *"in vitro"* and *"in vivo"* serum lipase levels, see references 6 and 7.

PROCEDURE NOTES

1. Absorbance measurement

a. Reagent Blank: If (A^0 - A^1) is a negative value, it should be considered as zero. However, it should normally be between 0.000 and 0.005.

b. Photometric instruments with relatively broad half-band widths (Coleman Jr II = nominal 20 nm) will give much lower absorbance readings than instruments with relatively narrow half-band widths (Turner-Sequoia = nominal 8 nm).

c. If the absorbance reading of the Working Reagent with the sample added is too high for photometer, you may use a 0.025% w/v Potassium dichromate Solution in 0.05 N sulfuric acid (or a dilution of this solution) to zero the instrument. Thus allowing the measurements to fall within the linear capability of the instrument.

1. Between 5-7% of patient sera show an increase in absorbance values rather than a decrease[8]. Also, an unexpectedly rapid decrease in lipase activity upon dilution of the sample may occasionally be observed. This phenomenon is referred to by Pope[9] as "pseudolipase" activity and is reportedly[10] due to the presence of rheumatoid factor and other disorders of the active immune processes (i.e., multiple myeloma, etc.). In case where this "pseudolipase" activity is encountered, the following procedure is recommended.

a. Add 0.2 ml (200 µl) of PEG Reagent to a 1.5 ml microcentrifuge tube.

b. Add 0.4 ml (400 µl) of patient sample, mix thoroughly and let stand at room temperature for 5 minutes.

c. Centrifuge for 2 minutes at 18,000 x g or for 10 minutes at 2,000 x g.

d. Assay for lipase according to the above procedure but use 150 µl (0.15 ml) of supernatant.

e. Calculate the lipase activity as above (the dilution factor has been compensated through the use of 0.15 ml (150 µl) of sample.

$$\frac{\text{Patient}(Ao - A1) - \text{Reagent Blank}\ (Ao - A1)}{\text{Reagent Blank}\ (Ao)} \times 3000 = \text{LIPASE ACTIVITY (U/L)}$$

2. An amylase assay is advisable whenever sample is encountered that demonstrates other than normal activity.

QUALITY CONTROL

For accuracy and precision check, we recommend use of normal and abnormal UDI controls based on human serum.

ORDERING INFORMATION:

UDITROL 'N' (Normal Serum Control) REF # 070N-010 2x5 ml
UDITROL 'A' (Abnormal Serum Control) REF # 070A-010 2x5 ml

OC 011/049. Issue05.Issued On Nov 2007 EDMA CODE 11.01.01.23.00

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PRODUCT AVAILABILITY

LIPASE (TURBIDIMETRIC / RATE)

REF # 049-021 2 x 30 ml

EC REP
(Authorized representative in the European Community)

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Wittekamp 30
D-30163 Hannover
Germany

CE