Leucine aminopeptidase Assay Kit (LAP)

Method: L-leucyl-nitroaniline Sbstrate

Cat .No.	Size	Instrument
GB8040G	R1: 2×60 ml R2: 2×20 ml	For Hitachi 7060/7150 & ShimadzuCL7200/8000
GS8041G	R1: 2×60 ml R2: 2×20 ml	For Hitachi 7170/7080 & Olympus AU640/400/600
GT8041G	R1:2×48 ml R2:2×16 ml	For TOSHIBA
GX8041G	R1:2×60 ml R2:2×20 ml	For SYNCHRON CX4-5-7-9/LX20/DXC600-800
GD8041G	R1: 24×3.8 ml R2:12×2.6 ml	For DATE DIMENSION

INTENDED USE

For the *in vitro* quantitative determination of Leucine aminopeptidase (LAP) in human serum, plasma and urine.

CLINICAL SIGNIFICANCE

Leucine aminopeptidase is metallopeptidase that cleave N-terminal residues from proteins and peptides. It is found in many tissues, but the highest levels are found in liver and gallbladder. Tissues destruction leads to the release of the intracellular enzyme into the circulating blood. Elevated serum LAP levels may be found in liver disease (such as hepatitis, cirrhosis and liver cancer), biliary disease, pregment. Elevated urine LAP levels may be found in kidney disease.

ASSAY PRINCIPLES

Leucine aminopeptidase catalyzes the hydrolysis of L-leucine-p-nitroanilide to L-leucine and p-nitroaniline. The rate of formation of p-nitroaniline is determined by the rate of increase in absorbance at 405 nm.

$$\text{L-leucine-p-nitroanilide} + H_2O \xrightarrow{\text{LAP}} \text{L-leucine} + \text{p-nitroaniline}$$

SAMPLE COLLECTION AND PREPARATION

Serum, heparinized plasma, or urine.

REAGENT COMPOSITION

Contents	Concentration of Solutions
Reagent 1 (R1)	
Buffer	100 mmol/L
Reagent 2 (R2)	
L-leucine-p-nitroanilide	15 mmol/L

STABILITY AND PREPARATION OF REAGENTS

All reagents are ready to use.

Stable up to the expiry date when stored at 2-8℃. The LAP assay kit reagents is stable for 10 days after opening and on-board the analyser at 2-8°C.

ASSAY PROCEDURE

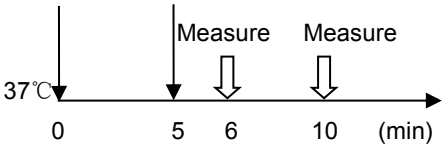
Test Procedure for Analyzers (HITACHI 917)

Assay Mode: Rate A 24-31

Wave Length (main/sub): 405 nm/660 nm

Sample:10 µl

R1: 180 µl R2: 60 µl



1. Mix 10 µl sample with 180 µl R1 and incubate at 37℃ for 5 minutes.
2. Add 60 µl R2 into cuvette, mix and incubate at 37℃ for 1 minute.
3. Read initial absorbance and start timer simultaneously, read again after 1, 2 and 3 minutes.
4. Calculate absorbance change per minute (ΔA/min)

CALCULATION

Calculation using calibration

$$\text{Concentration} = \frac{\Delta A_{sample}/\text{min}}{\Delta A_{calibrator}/\text{min}} \times \text{calibrator value}$$

Calculation using factor

$$\text{LAP (U/L)} = \frac{\Delta A/\text{mn} \times V_t}{\varepsilon \times V_s \times L} \times 1000 = \Delta A/\text{min} \times K$$

K= 2080

CALIBRATION

Recommend that this assay should be calibrated using Gcell Calibration.

QUALITY CONTROL

Randox Assayed Multisera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the

range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check reaction temperature.
3. Check expiration date of kit and contents.

REFERENCE VALUE [1]

Serum : 12-37 U/L

Urine: 2- 8 U/L

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

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SPECIFIC PERFORMANCE CHARACTERISTICS

LINEARITY
The method is linear up to 300 U/L. If the sample above this concentration should be diluted it with 0.9% NaCl and repeat assay. Multiply the result by dilution factor.

PRECISION
The CV of the test should be ≤5%

Intra assay precision		
N=20	Level1	Level 2
Mean (U/L)	17.6	15.1
SD	0.50	0.22
CV	2.86%	1.49%
Inter assay precision		
N=5	Level1	Level 2
Mean (U/L)	17.9	15.1
SD	0.32	0.21
CV	1.77 %	1.39 %

SENSITIVITY
The minimum detectable concentration of LAP with an acceptable level of precision was determined as 0.94 U/L

INTERFERENCE
The following analytes were tested up to the levels indicated and found not to interfere:

Hemoglobin:	500 mg/dl
Intralipid:	1000 mg/dl
Bilirubin:	50 mg/dl
Ascorbic Acid:	50 mg/dl

CORRELATION
This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:
y = 1.013x - 0.228, and a correlation coefficient of 0.998; 80 patient samples were analyzed.

SAFETY PRECAUTIONS AND WARNINGS
1. For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handing laboratory reagents.
2. The reagents contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.
3. Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.
4. All specimens used in this test should be considered potentially infectious. Universal Precautions, as they apply at your facility, should be used for handling and disposing of materials during and after testing.

REFERENCES
1. Hu Wangping, Hu Yingying, etc. The diagnostic significance of serum leucine aminopeptidase for liver and biliary disease, Journal of Clinical Hepatoplogy，2004,7 (1):35 -36.
2. Goldbarg JA, Rutenberg AM. The colorimetric determination of leucine aminopeptidase in urine and serum of normal subjects and patients with cancer and other diseases. Cancer 1958, 11: 283 - 291

INDEX OF SYMBOLS

	Manufacture
	Catalogue Number
	Lot number
	Date of manufacture
	Use by(Expiration date)
	For In-Vitro Diagnostic use only
	Stored at 2-8℃
	Attention:See instruction for use
	Authorized Representative in the European Company

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