CHRONOLAB

CE
IVD

# LAP

NAGEL. Kinetic

Quantitative determination of leucine aminopeptidase (LAP)

***PACKAGING***

| | |
|---|---|
| Ref.: 101-0551 | Cont.: 20 x 3 mL |

Store at 2-8ºC

***CLINICAL SIGNIFICANCE***

Leucine aminopeptidase (LAP) is a liver enzyme that has relatively broad specificity. Principally, serum LAP activity is increased in infants with jaundice caused by hepatocellular damage, in viral hepatitis, cirrhosis, hepatic neoplasms, acute pancreatitis and biliary obstruction[2,5,6].

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

***PRINCIPLE OF THE METHOD***

Leucine aminopeptidase (LAP) is a proteolytic enzyme hydrolysing the peptide bond adjacent to a free amino group.

It is called leucine amino peptidase as it rapidly catalyses the hydrolysis of leucine containing peptides, according the following reaction:

$$\text{L-Leucyl-p-nitroanilide} \xrightarrow{\text{LAP}} \text{L-leucine + p-Nitroanilide}$$

The rate of L-leucine formation, measured photometrically, is proportional to the catalytic concentration of LAP present in the sample[1].

***REAGENTS***

| | | |
|---|---|---|
| **R 1** Buffer | Phosphate pH 7.2 | 100 mmol/L |
| **R 2** Substrate | L-Leucyl-p-nitroanilide | 0.8 mmol/L |

**Optional (not included in the kit)**

| | | | |
|---|---|---|---|
| Contro-N | Ref.: 101-0252 | 4 x 5 mL | Lyophilized human control serum |
| | Ref.: 101-0083 | 20 x 5 mL | |
| Contro-P | Ref.: 101-0253 | 4 x 5 mL | Lyophilized human control serum |
| | Ref.: 101-0084 | 20 x 5 mL | |

***PREPARATION***

Working reagent (WR):

Dissolve (→) one tablet of R 2 Substrate in one vial of R 1 Substrate. Cap and mix gently to dissolve contents.

Stability:

7 days at 2-8º C or 48 hours at room temperature (15-25º C).

***STORAGE AND STABILITY***

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8º C, protected from light and contaminations prevented during their use.

Do not use the tablets if appears broken.

Do not use reagents over the expiration date.

**Signs of reagent deterioration:**

- Presence of particles and turbidity.
- Blank absorbance (A) at 405 nm ≥ 0.40.

***ADDITIONAL EQUIPMENT***

- Spectrophotometer or colorimeter measuring at 405 nm.
- Thermostatic bath at 25º C, 30° C o 37° C (± 0.1º C)
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

***SAMPLES***

Serum[1].

***PROCEDURE***

**Notes:** CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

1. Assay conditions:
   Wavelength: . . . . . 405 nm
   Cuvette: . . . . . .1 cm light path
   Constant temperature . . . . . .25º C / 30º C / 37º C
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:

| | |
|---|---|
| WR (mL) | 1.0 |
| Sample (µL) | 70 |

4. Mix.
5. After 1 minute, read initial absorbance (A) of the sample, start the stopwatch and read absorbances at 1 minute intervals thereafter for 3 minutes.
6. Calculate the difference between absorbances and the average absorbance differences per minute (ΔA/min).

***CALCULATIONS***

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|---|
| ΔA/min x 1544 = U/L of LAP |

**Units:** One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

***QUALITY CONTROL***

Control sera are recommended to monitor the performance of assay procedures.

If control values are found outside the defined range, check the instrument, reagents and technique for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

***REFERENCE VALUES[1]***

| | 25º C | 30º C | 37º C |
|---|---|---|---|
| Serum | 8-22 U/L | 12-33 U/L | 20-55 U/L |
| Urine | 0.6-4.7 U/L | 0.9-7 U/L | 1.5-11 U/L |

These values are for orientation purpose; each laboratory should establish its own reference range.

***PERFORMANCE CHARACTERISTICS***

**Measuring range:** Up to linearity limit of 0.250 ΔA/min.

If the results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl (9 g/L) and multiply the result by 10.

**Accuracy:** Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results of the performance characteristics depend on the analyzer used.

***INTERFERENCES***

Haemolysed serum samples should not be used[1].

A list of drugs and other interfering substances with LAP determination has been reported by Young et. al[3,4].



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***BIBLIOGRAPHY***

1. Nagel W et al. Klin Wschr 1964; 42: 446-449.
2. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 436.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
5. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
6. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.



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