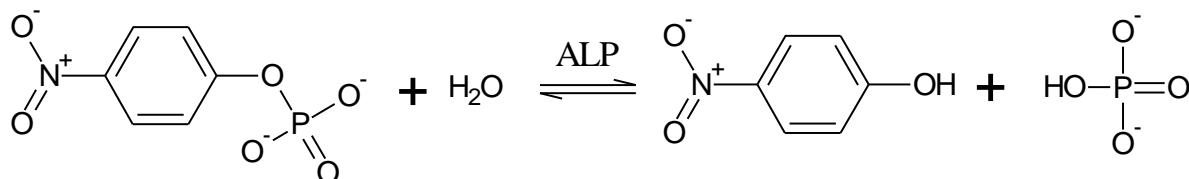


ALKALINE PHOSPHATASE

Principle:

Alkaline phosphatase (alkaline phosphohydrolase of orthophosphoric monoesters, E.C. 3.1.3.1. – ALP) splits 4-nitrophenylphosphate into 4-nitrophenol and phosphate in N-methyl 1-D-glucamine buffer. The enzyme activity is measured by the amount of the liberated 4-nitrophenol which can be determined by the constant time method using inhibitor blocking the enzyme active center.



Reagents:

- substrate: 92 mM 4-nitrophenylphosphate
- buffer: 0.43 M N-methyl-D-glucamine
- inhibitor: chelatone III, NaOH

Procedure:

test tube	sample	blank
buffer (ml)	2.00	2.00
serum (μl)	40	–
Mix and preincubate for 5 min at 37 °C. Then add:		
substrate solution (ml)	0.4	0.4
Mix and incubate for exactly 10 minutes at 37°C. Then add:		
inhibitor solution (ml)	1.0	1.0
serum (μl)	–	40

Mix well and measure the absorbances of both the sample and blank at the wavelength of 405 nm.

Calculation:

$$\text{Activity } (\mu\text{kat/l}) = 7.96 \times (A_{\text{sample}} - A_{\text{blank}})$$

Reference values:

- men: 0.9–2.3 $\mu\text{kat/l}$
- women: 0.74–2.1 $\mu\text{kat/l}$
- children (up to 14 years): 1.2–6.3 $\mu\text{kat/l}$

γ -GLUTAMYLTRANSFERASE

Principle:

The enzyme transfers the γ -glutamyl group from γ -L-glutamyl-3-carboxy-p-nitroanilide to glycylglycine, releasing 5-amino-2-nitrobenzoate. The enzyme activity is measured by the amount of the liberated 5-amino-2-nitrobenzoate which is determined spectrophotometrically at 410 nm.

Reagents:

- working reagent: 6.5 mM γ -glutamyl-3-carboxy-p-nitroanilide
- 165 mM glycylglycine

Procedure:

Pipette into a cuvette:

Working reagent (37°C)	1 ml
Set up the spectrophotometer, prepare the stopwatch. Then add:	
Serum	100 μ l

IMMEDIATELY mix, insert the cuvette into the photometer ($\lambda = 410$ nm), and record:

- initial absorbance
- absorbances at one-minute intervals thereafter for 3 minutes (use the stopwatch).

Calculation:

Calculate the differences between consecutive absorbances and the average absorbance difference per minute ($\Delta A/\text{min}$). γ -GT activity can be calculated using the formula

$$\text{activity } (\mu\text{kat/l}) = 23.19 \times (\Delta A/\text{min})$$

Reference values:

- men: 0.25–1.77 $\mu\text{kat/l}$
- women: 0.17–1.1 $\mu\text{kat/l}$