

Hydrolases (alkaline phosphatase)

Exercise 1

Phosphatase hydrolyzes disodium phenylphosphate. The amount of liberated phenol (the measure of enzyme activity) is determined colorimetrically with Folin-Ciocalteu reagent.

Unit of King-Armstrong is expressed in mg of phenol which is liberated from disodium phenylphosphate by enzyme in 100 ml blood plasma or plasma of semen in temperature 37°C during 15 minutes and pH=10 for alkaline phosphatase.

Protocol:

Test tube	0	1	2
Substrate for Alkaline phosphatase	2 cm ³	2 cm ³	2 cm ³
Blood Plasma	-	0.2 cm ³	0.2 cm ³

Incubation for 30 minutes in 37°C.

Blood Plasma	0.2 cm ³	-	-
Folin reagent	1.8 cm ³	1.8 cm ³	1.8 cm ³

The centrifugation in a laboratory centrifuge for 15 min.

Supernatantant	2 cm ³	1 cm ³	2 cm ³
15% Na ₂ CO ₃	2 cm ³	2 cm ³	2 cm ³

Incubation for 10 minutes in 37°C.

Read the absorbance of samples 0,1,2 against distilled water at a wavelength of 600 nm.

Calculations:

Calculation of enzyme activity:

$$\Delta E = E_B - E_K$$

$$\Delta E \times 1,6 \times 100 \times 7,09 = 1134,4 \times \Delta E = Iu/l$$

