

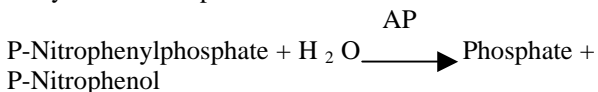


ALKALINE PHOSPHATASE (K) KIT

METHOD : PNPPMethod

PRINCIPLE:

The enzymatic reaction sequence employed in the assay of Alk-Phosphatase is as follows:



REAGENTS:

1. Reagent R1 : Buffered substrate – 20x1.2 ml
2. Reagent R2 : Buffer solution – 25 ml

REAGENT PREPARATION:

Reconstitute one vial with 1.2 ml of buffer solution as the working reagent.

COLLECTION AND HANDLING OF SPECIMEN:

1. Serum is the sample of choice. Avoid hemolysis.
2. Heparinized plasma may also be used. Oxalate, Fluoride and EDTA inhibit Alk-Phosphatase, hence unsuitable as Anticoagulants.
3. Perform the assay as soon as possible. Alk-Phosphatase in serum or plasma or in reconstituted control serum, rise significantly when stored.

PROCEDURE:

ASSAY PARAMETERS:

MODE	: Kinetics
REACTION SLOPE	: Increasing
WAVE LENGTH	: 405 nm
TEMPERATURE	: 37°C
OPTICAL PATH LENGTH	: 1 cm
BLANK	: Purified water
REAGENT VOLUME	: 1000 ul
SAMPLE VOLUME	: 20 ul
READ TIME	: 180 sec
DELAY	: 60 sec
DELTA	: 60sec
NUMBER OF READING	: 3
FACTOR	: 2757
LINEARITY	: 700 IU\ L
UNITS	: IU\ L

MANUAL ASSAY:

Pipette into cuvettes	Macro	Semi-Micro
Working Reagent	1000 µl	500 µl
Sample	20 µl	10 µl

Mix & take the first reading after 60 Sec. and take three additional readings at 60 Sec. intervals. Calculate mean absorbance change per minute (A/min)at 405nm

Note: If A/min exceed 0.250, dilute 1 Part of the sample with 9 parts of 0.9% of NaCl solution and re assay

Multiply the result by 10.

CALCULATIONS:

$$\text{IU/L} = \text{Abs./min} \times 2757$$

EXPECTED VALUES

Children: 180 – 1200 U/l 1.3 – 20 ukat/l
 Adults: 100 – 290 U/l 1.7 – 4,8ukat/l

LINEARITY

This unit is linearity upto 700 IU\ L

NOTE: The expected values are not to be confused with those indicated for AMP Method.

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