ALKALINE PHOSPHATASE

Histochemical Method 50 Tests

PRINCIPLE :

It is based on direct precipitation of lead phosphate at pH 9.5, the optimum pH for the enzyme activity, then converting it to dark brown precipitate of lead sulphide. The advantages of this method is the stability of incubation medium and the color of the end product and the well localized stain with different degrees of intensity , the absence of nuclear stain and the high sensitivity. The method is based on using tris as buffer ,L-tartarate as chelating agent for lead ions and B-glycerophosphate as substrate.

SAMPLES:

Frozen sections of different organs.
Fresh human blood films were used for demonstrating alkaline phosphatase activity in the leucocytes.

REAGENTS :

1.	Buffer-Substrate	100	ml
2.	Lead Nitrate	10	ml
3.	Magnesium Chloride	5	ml
4.	Yellow Ammonium Sulphid	20	ml
5.	Methyl Green	20	ml

Additional Reagents:

Formaline - methanol fixative

STABILITY:

The reagents are stable up to the expiry date specified when stored at +4 to +8 °C

PROCEDURE:

Fixation: by formaline-methanol.

- Frozen tissue sections are fixed for 30- 60min at 4 °C.
- Fresh blood film is fixed at R.T. for 2-5 min.
- Following fixation, sections were rinsed in distilled water before incubation.

Working Reagent :

Reagent 1	Buffer - Substrate	22.5 ml
Add dropwise with stirring		
Reagent 2	Lead Nitrate	2.5 ml
Reagent 3	Magnesium chloride	1.0 ml

Filter if necessary . The medium is stable for few hours at R.T. Appropriate volumes of working reagent may be prepared using the same ratio (2.25: 0.25 :0.1).

Incubation Method:

1. Incubation was carried out at 37°C . in the working reagent, 15-30 min. for blood smear and more or less for tissue according to the type of tissues.
2. Section was washed by distilled water, then covered for one minute in weakly alkaline ammonium sulphide solution R4 (10 drops) . Lead phosphate produced by the reaction was visualized as dark brown lead sulphide granules.
3. After washing with distilled water, section were dried and counter-stained with methyl green R5 (10 drops) for 20-30 min.wash with distilled water then dried, ready for examination with oil immersion lens.

- Section immersed in boiling water for one minute were used as negative control.
- Blood smear for normal healthy subject should be used as normal positive control.

Scoring Technique:

Scoring of leucocytes alkaline phosphatase activity was carried out by using the oil immersion lens, 100 consecutive segmented and band neutrophils were rated from 0 to +4 on the bases of the quantity and intensity of the reaction within the cytoplasm of these cells. The sum of ratings of the 100 cells was regarded as the "total scors" for a given blood smears.
The score ratings was based on the following criteria:

Score zero:	Colorless cytoplasm.
Score one:	Rare fine dark brown granules confind to small area of the cytoplasm .
Score two:	Some fine and moderately coarse granules unevenly distributed through half of the cytoplasm .
Score three:	Moderately coarse granules distributed throughout the greater part of the cytoplasm.
Score four:	Coarse granules distributed throughout the whole cytoplasm, and partially obscuring the nucleus.

See Table and figs as reference.

Results:

Alkaline Phosphatase activity: Brownish black
Nuclei: Green

REFERENCE :

EL-Aaser A.A., Hindawy ,D.S ,Hammouda, F-Mohieddin,O.Acta Biol Acad .Sci. Hung (1977) ,28,291

BIODIAGNOSTIC
DIAGNOSTIC AND RESEARCH REAGENTS

ALKALINE PHOSPHATASE
Histochemical Method

+4 to +8 ºC 50 Tests

For Diagnostic and Research use

CAT. NO. AP 26 10

REAGENTS

R1	Buffer - Substrate	**100 ml**
R2	Lead Nitrate	**10 ml**
R3	Magnesium Chloride	**5 ml**
R4	Yellow ammonium Sulphide	**20 ml**
R5	Methyl Green	**20 ml**

CONTACTS

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