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ALKALINE PHOSPHATASE STAINING PROTOCOL

PRINCIPLE:

Alkaline phosphatase is a generic name for phosphomonoesterases that hydrolyze orthophosphate at an alkaline pH. These enzymes are widely distributed, usually activated by magnesium, manganese, zinc, and cobalt ions and inhibited by cysteine, cyanides, and arsenates. This is a simultaneous coupling azo dye method first developed in 1944. It has been modified repeatedly. Sodium α-naphthyl acid phosphate, the substrate used in this protocol, is hydrolyzed and then coupled to the diazonium salt (Fast Blue RR) which is then precipitated at the site of enzyme activity.

SPECIMEN REQUIRED:

Snap frozen human striated muscle.

Controls:

Stain several different muscles simultaneously. Endothelium in small arterioles (less than 80 μm) and larger endomysial capillaries normally stains positive.

METHOD

Fixation: None, use snap frozen tissue.

Technique: Cut 10 - 16 micron (12 µm) sections in cryostat from snap frozen biopsy. Attach one or more sections to a Superfrost Plus Microscope Slide.

Equipment:

Coplin staining jar - Thomas Scientific #8541L10
Forceps Latex gloves

Reagents:

Glacial Acetic Acid -Fisher A507-500, **CORROSIVE**
Store at room temperature
Fast Blue RR salt - Sigma F0500 Store at -20 desiccated
Alpha-napthyl acid phosphate, monosodium salt ($C_{10}H_8O_4PNa$)-
Sigma N7000
Sodium barbital (5,5' dietyl barbituric acid) - Sigma B0500,
NARCOTIC, TOXIC,CONTROLLED SUBSTANCE
Store at room temperature

Solutions:

I. 0.1 M Sodium Barbital Solution

5.15 gm Barbital powder (room temp.) + deionized $H_2O \rightarrow$ 250 ml

II. 1 % Acetic Acid: 1 ml Glacial acetic acid to deionized $H_2O \rightarrow$ 100 ml

III. 1 N NaOH

IV. Incubating Solution (prepare fresh for each stain)

15 ml 0.1 M Sodium Barbital Solution
15 mg Sodium Alpha-Napthyl Acid Phosphate
15 mg Fast Blue RR salt (a fine brown precipitate will form)
Adjust pH to 9.2 with 0.1 N HCl (~ 4 to 5 drops)
Filter solution just prior to use

Staining Procedure

1. Place slides in the incubating solution in a Coplin staining jar (Thomas Scientific #8541L10) for 60 minutes at room temperature.

2. Wash with three exchanges of tap or deionized H_2O.

3. Place in 1 % Acetic Acid for 10 minutes.

4. Rinse with deionized water, 2 to 3 changes.

5. Let air-dry for at least one hour (overnight is better).

6. Rehydrate with deionized water (approximately 10 minutes).

7. Clean back of slides with cotton swab.

5. Mount with aqueous medium (e.g. Glycerogel).

Results

Sites of alkaline phosphatase activity are localized as a fine precipitate colored Black/Dark-blue.

REFERENCES

1. Barka and Anderson, **HISTOCHEMISTRY**, Harper & Row, New York, 1963.

2. Sheehan and Hrapchak, **HISTOTECHNOLOGY**, 2nd Edition; Batelle Press, Columbus, 1987.

3. Thompson, **SELECTED HISTOCHEMICAL AND HISTOPATHOLOGICAL METHODS**, Charles C. Thomas, Springfield, IL, 1966.

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