Washington University School of Medicine
Neuromuscular Lab
CAP: 1923316
CLIA: 26D0652044
NY: PFI 3499

ACID PHOSPHATASE PROTOCOL

PRINCIPLE:

The complex naphthol, naphthol acid phosphate, is hydrolyzed by acid phosphatases present in the tissue, and napthol derivatives are therby produced. The naphthol derivatives couple with the unstable diazonium salt, hexazonium pararosanilin, to produce a red azo dye to mark the site of enzyme activity.

SPECIMEN REQUIRED:

Snap frozen human striated muscle.

Controls:

Stain several different muscles simultaneously. There is often enzyme activity associated with lipofuscin in muscle. The degree and location of staining has pathologic interest.

METHOD:

Fixation: None. Use snap frozen tissue.

Technique: Cut 10 - 16 micron (12 µm) sections in cryostat from snap frozen tissue. Attach one or more sections to a No.1½, 22 mm square coverslip.

Equipment:

Ceramic staining rack - Thomas Scientific #8542-E40
Columbia staining dish - Thomas Scientific #8542-C12
Columbia staining dish(jar) - Thomas Scientific #8542-E30
Forceps, Latex gloves

Reagents:

Calcium Chloride, anhydrous - Sigma C4901,
Store at room temperature
Formaldehyde, 37 % - Fisher F79-500,
POISON, CARCINOGEN, Store at room temperature
Hydrochloric acid, ACS - Fisher A144-500,
CORROSIVE, Store at room temperature
Alpha-napthyl acid phosphate, monosodium salt ($C_{10}H_8O_4PNa$)-
Sigma N7000
Basic Fuchsin- Santa Cruz 203731 (troubleshooting: RC; 30/172)
Permount - Fisher SP15-100, **FLAMMABLE HEALTH HAZARD**
Reagent alcohol, ACS,- histochemical Fisher A962-4 or HPLC A995,
FLAMMABLE, TOXIC, TERATOGENIC,
Store at room temperature in flammable cabinet
Sodium acetate, trihydrate - Sigma S9513, Store at room temperature
Sodium barbital (5,5' dietyl barbituric acid) - Sigma B0500,
NARCOTIC, TOXIC,CONTROLLED SUBSTANCE
Store at room temperature
Sodium nitrite certified crystalline - Fisher S347 -
STRONG OXIDIZER, COMBUSTIBLE
Xylenes - Fisher #HC700-1GAL, **FLAMMABLE**
Store at room temperature in flammable cabinet.

I. Barbital Acetate Solution

Sodium barbital(sodium barbiturate)	1.47 g
Sodium acetate($NaC_2H_3O_2 \cdot 3\ H_2O$)	0.97 g
deionized water -> final volume of 50 ml	

II. Basic Fuchsin Solution (Store at 4^{o}C)

Basic Fuchsin Hydrochloride (C.I. 42500)	0.5 g
deionized water	10.0 ml
Concentrated Hydrochloric Acid	2.0 ml

Dissolve dye in water, add acid, heat gently, cool to room temp & filter .

III. Sodium Nitrite, 4% (w/v) (Store at 4^{o}C) Store at 4^{o}C

Sodium Nitrite ($NaNO_2$)	0.4 g
deionized water	10.0 ml

IV. Baker's Solution (modified)

Calcium Chloride (anhydrous) $CaCl_2$	0.3 g
Formaldehyde, 37%	3.0 ml
deionized water -> final volume of 100 ml	

V. Indicator Solution:

Basic Fuchsin HCl Solution	0.4 ml
Sodium Nitrite Solution, 4%	0.4 ml
mix well, let stand for ~30 secs.	

VI. Incubating Solution:

Sodium alpha napthyl acid phosphate	20 mg
Barbital Acetate Solution	5.0 ml
deionized water	13.0 ml
Indicator Solution (V)	0.8 ml
Adjust pH to 5.6 to 5.8 (5.7) with HCl (1.0 and 0.1 N)	

VII. Alcohol 50 %

reagent alcohol	~50 ml
deionized water	~50 ml

VIII. Alcohol 70 %

reagent alcohol	~70 ml
deionized water	~30 ml

IX. Alcohol 80 %

reagent alcohol	~80 ml
deionized water	~20 ml

X. Alcohol 95 %

reagent alcohol	~95 ml
deionized water	~ 5 ml

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Staining Procedure

1. Place coverslips with sections in Baker's Solution in a Columbia staining dish (Thomas Scientific #8542-C12) for 5 minutes at room temperature.

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

3. Add incubation solution and stain for at least one (2) hour at room temperature in a dark place.

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

5. Dehydrate (fairly rapidly) in ascending alcohols (50%,70%,80%,95% x 2, 100% x 2) in ceramic staining rack - Thomas Scientific #8542-E40.

6. Clear with at least 2 changes of xylene also in a ceramic coverslip rack - Thomas Scientific #8542-E40

7. Mount with PERMOUNT or another synthetic organic mounting medium.

Results:

Red azo dye indicates sites of acid phosphatase activity.

REFERENCES:

1. Barka, T., 1961. In: *SELECTED HISTOCHEMICAL AND HISTOPATHOLOGICAL METHODS,* S.W. Thompson; Charles C. Thomas, Springfield, IL, 1966.

2. Barka, T. and Anderson, P.J., 1962. In: *THEORY AND PRACTICE OF HISTOTECHNOLOGY*, Sheehan, D.C. and Hrapchak, B.B., 2nd Edition1980;Battelle Memorial Institute, Columbus, OH, 1987.

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