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

ESTERASE STAINING: ALPHA-NAPTHYL ACETATE PROTOCOL

PRINCIPLE:

This protocol demonstrates the sites of non-specific esterases in tissue sections. This modification of the technique described by B. J. Davis demonstrates small, angular denervated muscle fibers and neuromuscular junctions

SPECIMEN REQUIRED

Snap frozen human striated muscle

METHOD

Fixation: None. Use snap frozen tissue

Technique: Cut 10 -16 micron (12 micron) sections in cryostat from snap frozen biopsy. Attach one or more sections to a No. 1 ½ 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
Filter paper (Baxter #f2217-070, Grade 363 Qualitative)

Reagents:

Acetone - Baxter #010-4 **FLAMMABLE**
Alpha-naphthyl acetate - Sigma N8505 - Store desiccated at -20°C
deionized water
Hydrochloric acid, ACS - Fisher A144-500, **CORROSIVE**,
Store at room temperature
Basic Fuchsin- Santa Cruz 203731 (troubleshooting: RC;30/172),
Store at room temperature
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 nitrite certified crystalline - Fisher S347 **or** Sigma S2252,
STRONG OXIDIZER, COMBUSTIBLE
Sodium dibasic phosphate (Na_2HPO_4) anhydrous, ACS (FW 141.96)
Store at room temperature
Xylenes - Fisher #HC700-1GAL, **FLAMMABLE**,
Store room temperature in flammable cabinet)

Solutions:

1. 0.2 M Sodium Phosphate
   Sodium phosphate dibasic, anhydrous (Na_2HPO_4) 7.1 g
   deionized water → 250 ml
   Store at room temperature

2. 4% Basic Fuchsin HCl
   Dissolve Basic Fuchsin 0.5 g
   in deionized water 10 ml
   Heat gently on a hot plate **(DO NOT BOIL)**
   Add concentrated (12N) hydrochloric acid 2.5 ml
   Cool to room temperature
   Filter (Baxter #f2217-070, Grade 363 Qualitative)
   Store refrigerated (0-5^oC)

3. 4% Sodium Nitrite
   Sodium nitrite ($NaNO_2$) 0.5 g
   deionized water 12.5ml
   Store refrigerated (0-5^oC)

4. "*Azotized Basic Fuchsin*" **(PREPARED FRESH FOR EACH STAIN)**
   4% Basic Fuchsin -HCl (Solution #2) 0.2 ml
   4% sodium nitrite (Solution #3) 0.2 ml
   Sit at room temperature for a few minutes. (Amber color solution.)

5. Staining Solution (**PREPARED FRESH FOR EACH STAIN)**

   Into a 30 ml glass beaker **ADD IN THE ORDER STATED**
   Alpha-naphthyl Acetate ~ 2 mg
   Acetone ~ 0.75 ml
   MIX WELL
   Add 0.2 M sodium phosphate (Na_2HPO_4) 12.5 ml
   (*SOLUTION MAY BECOME CLOUDY - OK!)*
   MIX WELL

   Add Solution #4 ("*Azotized Basic Fuchsin")*
   and *MIX WELL*
   SOLUTION WILL CHANGE COLOR FROM YELLOW TO RED-BROWN IN LESS THAN FIVE (5) MINUTES !

6. Alcohol 50 %
   reagent alcohol ~50 ml
   deionized water ~50 ml

7. Alcohol 70 %

reagent alcohol	~70 ml
deionized water	~30 ml

8. Alcohol 80 %

reagent alcohol	~80 ml
deionized water	~20 ml

9. Alcohol 95 %

reagent alcohol	~95 ml
deionized water	~ 5 ml

Staining Procedure

1. Place coverslips into a Columbia staining dish (Thomas Scientific #8542-012)

2. When the Staining Solution (Solution %) is gold to red-orange in color, add it to the coverslips in the staining dish for 5 minutes at room temperature.

3. Immediately place sections under running tap water for several minutes to wash the reaction product off the sections.

4. Clean back of coverslips with cotton swab.

5. Place coverslips with sections in a ceramic rack (Thomas Scientific #8542-E40).

6. Dehydrate in ascending alcohol solutions (50%, 70%, 80%, 95% x 2, 100% x2) - in Columbia staining dishes - Thomas Scientific #8542-E30.

7. Clear with xylene (x - 4x) also in Columbia staining dishes - Thomas Scientific #8542-E30.

8. Mount coverslip onto a labeled glass slide with Permount or other suitable organic mounting medium.

Results

Esterase activity is demonstrated in denervated fibers as a red-brown color. Normal fibers exhibit a pale yellow to brown color. Neuromuscular junctions are demonstrated by a dark red-brown deposit on the edge of muscle fibers.

REFERENCES

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