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GOMORI TRICHROME STAIN PROTOCOL

PRINCIPLE:

Gomori's one-step trichrome is a staining procedure that combines the plasma stain (chromotrope 2R) and connective fiber stain (fast green FCF) in a phosphotungstic acid solution to which glacial acetic acid has been added.

SPECIMEN REQUIRED:

Snap frozen human striated muscle. (Use the isopentane freezing method previously described.)

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 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:

Glacial Acetic Acid -Fisher A507-500,
CORROSIVE store at room temperature
Chromotrope 2R - Sigma C3143,
IRRITANT, store at room temperature
Deionized water
Fast Green FCF - certified, Sigma F7258,
GLOVES AND MASK REQUIRED, store at room temperature
Harris Hematoxylin Stain, acidified, - Lerner Laboratories * #1931382
Store at room temperature
Permount - Fisher SP15-100, **FLAMMABLE HEALTH HAZARD**
Phosphotungstic acid, free acid, - Sigma P4006,
CORROSIVE, store at room temperature

Reagent alcohol, ACS - histochemical Fisher A962-4, or HPLC A995
FLAMMABLE, TOXIC, TERATOGENIC,
Store at room temperature in flammable cabinet
Xylenes - Fisher #HC700-1GAL,
FLAMMABLE, Store room temperature in flammable cabinet.

Solutions:

I. **Gomori's trichrome stain**

Chromotrope 2R	0.6 g
Fast green FCF	0.3 g
Phosphotungstic acid	0.6 g
deionized water	100 ml
Acetic acid, glacial	1.0 ml

Adjust pH of the above mixture to 3.4 using 1 N NaOH
Store at room temperature, **pH weekly**.

2. **Acetic acid, ~0.2 %**

deionized water	1000 ml
acetic acid, glacial	2 ml

3. **Alcohol 50** %

reagent alcohol	~50 ml
deionized water	~50 ml

4. **Alcohol 70 %**

reagent alcohol	~70 ml
deionized water	~30 ml

5. **Alcohol 80 %**

reagent alcohol	~80ml
deionized water	~20 ml

6. **Alcohol 95 %**

reagent alcohol	~95 ml
deionized water	~ 5 ml

Staining Procedure:

1. Place the coverslip with section in a ceramic staining rack (Thomas Scientific #8542-E40).
2. Immerse sections in Harris Hematoxylin for 1.5 minutes.
3. Wash with tap water until the water is clear.
4. Immerse sections in Gomori trichrome stain for 10 minutes.

5. Differentiate using 0.2% acetic acid. A few dips should be sufficient.

6. Immerse rack with sections directly into 95 % alcohol

7. Continue to dehydrate in ascending alcohol solutions (95% x 2, 100% x 2) in Columbia staining dishes - Thomas Scientific #8542-E30.

8. Clear with xylene (3 - 4 x) also in columbia staining dishes - Thomas Scientific #8542-E30.

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

Results:

Nuclei → red-purple
Normal muscle fibers → green-blue
Intermyofibrillar muscle membranes & mitochondria → red
Interstitial collagen → green

REFERENCES:

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

2. Sheehan, D.C. and Hrapchak, B.B. *THEORY AND PRACTICE OF HISTOTECHNOLOGY,* Battelle Memorial Institute, Columbus, OH, 1987.

12/26/2006