
ELASTIC TISSUE- MODIFIED ELASTIC TRICHROME STAIN

PURPOSE: To differentiate different types of connective tissue, in a single tissue section.

PRINCIPLE: Verhoff's Hematoxylin stains the elastic fibers, and a modified Masson's Trichrome is used to stain the other connective tissue elements.

FIXATIVE: 10% formalin or Zenker's

TECHNIQUE: Cut paraffin sections 4-5m.

EQUIPMENT: Microwave, coplin jars (rinse in DI water)

REAGENTS:

Weigert's Iodine:

See Microwave Trichrome

5% Hypo

See Stock Solutions

Zenker's Fluid:

Distilled water	500.0 ml
Mercuric chloride	25.0 gm
Potassium dichromate	12.5 gm
Sodium sulfate	5.0 gm

Mix well, label with date and initial. Solution is stable indefinite.

Working Solution: Right before use, to 50 ml of Zenker's add 5 ml glacial acetic acid. Discard after use.

CAUTION: Corrosive poison, discard in labeled glass container.

Verhoeff's Hematoxylin:

5% Alcoholic hematoxylin	20.0 ml
10% ferric chloride	8.0 ml
Lugol's iodine	8.0 ml

Add in order given, make right before use, discard when stain is completed.

Caution: Flammable, avoid contact and inhalation.

Biebrich Scarlet:

See Masson's Trichrome

Phosphotungstic

/phosphomolybdic Acid:

See Masson's Trichrome

Aniline Blue:

See Masson's Trichrome

1% Acetic Acid:

See Masson's Trichrome

CONNECTIVE TISSUE

MODIFIED ELASTIC TRICHROME STAIN

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SAFETY: Wear nitrile gloves and lab coat, wear a particle mask or work under the hood. Keep all hot uncapped solutions under the hood. Avoid contact and inhalation.

Zenker's contains; Mercuric chloride: Severe skin and eye irritant; target organ effects on reproductive, urogenital, respiratory, gastrointestinal and fetal systems via ingestion and inhalation routes.

Potassium dichromate: toxic by inhalation of dust and by ingestion; target organ effects on reproductive and fetal systems. Solid form is corrosive to eyes, skin and mucous membranes.

Iodine: dermal sensitizer, irritant to eyes, skin and respiratory system.

Sodium thiosulfate: Toxic on ingestion. Can irritate the stomach. Irritant to skin, eyes and respiratory tract.

PROCEDURE:

1. Deparaffinize and hydrate to water.
2. Mordant in Weigert's iodine, microwave 45 seconds.
3. Rinse in water.
4. Decolorize in 5% hypo solution, wash in water.
5. Mordant in Zenker's, microwave 60 seconds.
6. Wash in tap water.
7. Verhoeff's hematoxylin for 20-30 minutes.
8. Wash in water.
9. Hypo for 3-5 minutes.
10. Wash in water.
11. Biebrich scarlet, microwave 45 seconds.
12. Rinse in distilled water.
13. Phosphotungstic/phosphomolybdic acid, microwave 45 seconds.
14. Distilled water, quick dip.
15. Aniline blue, microwave 10 seconds.
16. Rinse in distilled water.
17. Acetic acid rinse for 1 minute.
18. Dehydrate quickly through alcohols, clear in xylene and coverslip.

RESULTS:

Elastic fibers and nuclei: black

Collagen: blue

Cytoplasm, muscle fibers, keratin, and fibrin: red

CONNECTIVE TISSUE

MODIFIED ELASTIC TRICHROME STAIN

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REFERENCES:

University of Utah Histology Laboratory S Q

Modified Movat's Stain.

Crookham,J, Dapson,R, Hazardous Chemicals in the Histology Laboratory,
2nd ED, 1991, Anatech

Prepared: _____ By: _____

Approved: _____ By: _____

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PROCEDURE CARD

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RESULTS:

cytoplasm, muscle fibers, keratin, fibrin: red collagen: blue
Elastic fibers, nuclei: black

SAFETY: Wear gloves and lab coat, work under the hood or in a well ventilated room. Do not discard Zenkers fix down the drain, it contains mercury. Dispose through Safety Services.

Weigert's Iodine:

See Microwave Trichrome

1% Acetic Acid:

See Masson's Trichrome

5% Hypo

See Stock Solutions

Verhoeff's Hematoxylin:

Alcoholic hematoxylin 20.0 ml

10% ferric chloride 8.0 ml

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Discard after use.

Biebrich Scarlet:

See Masson's Trichrome

CAUTION: Corrosive, poison

Phosphotungstic/

phosphomolybdic Acid:

See: Masson's Trichrome

Aniline Blue:

See Masson's Trichrome

ZENKERS FLUID:

Distilled water	500.0 ml
Mercuric chloride	25.0 gm
Potassium dichromate	12.5 gm
Sodium sulfate	5.0 gm

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Working Solution:

Zenkers	50.0 ml
Acetic acid	5.0 ml

Mix right before use. Discard after use.

CAUTION: Corrosive poison, discard in labeled glass containers

DATE: _____

TECH: _____

EXPIRATION: _____