
FONTANA-MASSON SILVER METHOD - MELANIN

PURPOSE: To identify argentaffin granules and melanin. Melanin is a nonlipid, nonhematogenous pigment. It is A brown-black pigment present normally in the hair, skin, retina, iris and certain parts of the CNS. Argentaffin granules are found in carcinoid tumors.

PRINCIPLE: A positive argentaffin reaction means the cells take-up silver and then reduce it to a visible metallic state, without the aid of a reducing agent.

CONTROL: A nevi for melanin, or small intestine for argentaffin.

FIXATIVE: 10% formalin

TECHNIQUE: Cut paraffin sections 4 μ .

EQUIPMENT: Acid cleaned glassware, microwave oven (or 60°C oven), microscope.

REAGENTS:

Ammoniacal Silver

Stock Solution:

10% Silver nitrate 25.0 ml

Add ammonium hydroxide drop by drop, until solution precipitates and clears again.

10% silver nitrate 1.0 ml

Solution will become slightly cloudy, allow to remain undisturbed for 4-24 hours. Discard after use.

CAUTION: CORROSIVE - avoid contact and inhalation.

Ammoniacal Silver

Working Solution:

Ammoniacal silver
stock solution 12.5 ml
Distilled water 37.5 ml

Pipette the ammoniacal silver off the top, leaving the precipitate on the bottom. Filter. Discard after use.

CAUTION: CORROSIVE - avoid contact and inhalation.

MINERALS AND PIGMENTS

FONTANA-MASSON

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10% Silver Nitrate:

Silver nitrate 20.0 gm
Distilled water 200.0 ml

Mix well, store in acid cleaned brown bottle, in the refrigerator. Good for 6 months.

CAUTION: Avoid contact and inhalation.

0.1% Gold Chloride:

1% Gold Chloride, Stock 5.0 ml
Distilled water 45.0 ml

CAUTION: Avoid contact and inhalation.

5% Hypo:

CAUTION: Avoid contact and inhalation.

Nuclear-Fast Red:

See Retic

SAFETY: Wear gloves, goggles and lab coat. Avoid contact and inhalation.

Silver nitrate: severe skin and eye irritant. Oxidizer. Ingestion will produce violent gastrointestinal discomfort. Possible carcinogen: equivocal tumorigenic agent.

Ammoniacal silver solution can be explosive, discard down the drain followed by copious amounts of running water.

Ammonium hydroxide: severe eye irritant, irritating to respiratory system. Target organ effects on respiratory system. Corrosive. Work under hood.

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

PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. *Working Silver Solution, microwave 20 power, 2 minutes. Check slides microscopically for adequate intensity, place in microwave a few seconds longer if needed.
3. Rinse in distilled water.
4. 0.1% Gold chloride, 10 minutes.
5. Rinse in distilled water.
6. 5% Hypo, five minutes.
7. Wash in tap water, rinse in distilled.
8. Nuclear-fast red, 5 minutes.
9. Wash in tap water.
10. Dehydrate, clear, and coverslip.

*Conventional Method: Place in 60°C oven for 1 hour.

RESULTS:

Melanin, argentaffin cells black
Nuclei red

REFERENCES:

- Sheehan D, Hrapchak B, Theory and practice of Histotechnology, 2nd Ed, 1980, pp 221,226-227, Battelle Press, Ohio
Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: _____ By: _____

Approved: _____ By: _____

PROCEDURE CARD

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10% Silver Nitrate:

Silver nitrate	20.0 gm
Distilled water	200.0 ml

Mix well, store in acid cleaned brown bottle, in the refrigerator. Good for 6 months.

CAUTION: Avoid contact and inhalation.

0.1% Gold Chloride:

1% Gold Chloride, Stock	5.0 ml
Distilled water	45.0 ml

CAUTION: Avoid contact and inhalation.

5% Hypo:

CAUTION: Avoid contact and inhalation.

Nuclear-Fast Red:

See Retic

10% SILVER NITRATE:

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Distilled water 200.0 ml

Mix well, store in acid cleaned brown bottle, in the refrigerator. Good for 6 months.

CAUTION: Avoid contact and inhalation.

DATE: _____

TECH: _____

EXPIRATION: _____

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EXPIRATION: _____

0.1% GOLD CHLORIDE

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TECH: _____

NUCLEAR-FAST RED

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TECH: _____